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CHEMICAL AND MICROSCOPICAL AIDS
TO CLINICAL DIAGNOSIS

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CHEMICAL AND
MICROSCOPICAL AIDS TO
CLINICAL DIAGNOSIS

BEING A GUIDE TO URINARY, GASTRIC, AND OTHER
ANALYSES EMPLOYED IN PRACTICAL MEDICINE

FOR THE USE OF STUDENTS AND PRACTITIONERS

BY

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Illustrated

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PREFACE.

THIS little book is intended to give, within a reasonably small compass, a practical description of those chemical and microscopical methods which are now so much employed as necessary adjuncts to clinical diagnosis. While every endeavour has been made to bring the book thoroughly up to date, I have avoided a multiplicity of tests and reactions, and have given as a rule only those which time and experience have proved to be of real value. An attempt has also been made to give the *rationale* of the various methods, so that the student may know not only the *how* and the *when*, but also the *wherefore* of the reactions and methods described.

Naturally the examination of the urine occupies the largest part of the subject-matter, but a sufficiently full description of the chemical analysis of gastric fluids and of the microscopical examination of the blood has also been given, and briefer accounts of the investigations concerned with the saliva and sputum, fæces, and pathological fluids. Few details of bacteriological methods have been included within the scope of this book, as I am of opinion that these can be much more satisfactorily obtained from a good modern text-book devoted to this special subject.

A certain number of references have been made in the text to original papers, and at the end of each section a short bibliography has been appended for the use of those who may desire to consult larger and more advanced text-books.

While intended primarily for the use of students of medicine, I hope that this book may also prove of use to those already engaged in practice; many men feel the need of a small yet sufficiently comprehensive volume of this kind, which it is hoped will meet all the demands likely to be made upon it, as far at least as everyday questions of chemical and microscopical analysis in practical medicine are concerned.

I take this opportunity of thanking Dr. Jas. Finlayson and Dr. John Love for useful suggestions in the selection of the contents, and Dr. R. S. Thomson for his kindness in reading over the proof-sheets. I also desire to acknowledge the courtesy of the following publishers in granting me the loan of various figures in the text: Messrs. Smith, Elder & Co. for figs. 11, 12, 14, 15, 17, 18, 19, and 21; Messrs. Cassells & Co. for figs. 10, 22, 24, and 33; Messrs. Longmans, Green & Co. for figs. 1, 8, and 9; Messrs. Charles Griffin & Co. for fig. 20; and Mr. Young J. Pentland for figs. 30 and 31.

C. C. D.

2 ROYAL CRESCENT,
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SECTION I.

THE URINE.

CHAPTER I.

The urine as a whole—Its relation to general metabolism—Collection of samples—Colour—Odour—Specific gravity—Reaction—Character of deposit—Quantity secreted—Reagents and apparatus required for urinary analysis—Table of weights and measures.

The urine as a whole. The kidneys are entitled to hold the first place as eliminators from the system of those substances which are most likely to prove harmful to the organism. Upon them devolves almost the entire task of removing the products of tissue-metabolism in the widest sense; they often eliminate poisons absorbed from the intestinal tract, and they must also pass through their substance many drugs and other bodies introduced into the system from without. This being so, we can readily understand how important a matter the chemical examination of the urine is, and, since its composition is obviously directly affected by the metabolic processes of the whole organism, what valuable information such examination may yield us both in health and disease.

The urine must always be looked upon as a complex whole, and regard must be paid not only to any single ingredient, but to that ingredient in relation to the other bodies that accompany it in the excretion. Some diseases modify but one element, others several. One morbid condition may lead to the appearance of a single pathological constituent, another may produce the development of several. The urinary excretion is never to be regarded as a constant; it varies frequently even in health, by day and by night, in fasting and in eating, during exercise and during sleep.

Collection of samples. This being the case, it is necessary that, whenever possible, the chemical examination should be carried out on a sample of the whole urine for twenty-four hours. This is advisable in the case of qualitative tests and is absolutely essential for quantitative work.

To collect a twenty-four hours' sample, let the patient make water at 8 A.M., and let this sample be rejected. All that is passed after this up to and inclusive of 8 A.M., on the following morning is kept. The total twenty-four hours' secretion is now well mixed, care being taken that any sediment that may have fallen is well shaken up and disseminated through the fluid. The total quantity is now measured and a sample of the mixed urine removed for examination. The sample taken should be kept in a cylindrical glass vessel called a urine-glass, 17 cm. high, about $4\frac{1}{2}$ cm. in diameter, and tapering in its lower fourth to a point, for the better collection of sediments. Such a vessel, if graduated, may be used as a measure. A slip of glass or other suitable covering should be placed over

it when filled, to exclude dust, and it should then be set aside for a few hours to allow any deposit to settle down. In cases where it is impossible to obtain a sample of the whole day's urine, it is best to examine that passed at eleven in the forenoon, or two and a half to three hours after a meal.

If it is desired to examine the sediment at once with the microscope, a portion of the sample may be centrifugalized. In many hospitals a hand-centrifuge is provided in the test-room, and is found to be of special use in obtaining sediments containing bacteria, blood-corpuscles, and tube-casts.

Having obtained a suitable sample, attention must now be directed towards certain points which we shall consider in detail; they are (*a*) colour, (*b*) odour, (*c*) specific gravity, (*d*) reaction, (*e*) character of deposit, and finally (*f*) a note should be taken of the total amount passed.

(*a*) COLOUR. Normally this is of a transparent amber or straw tint, but may vary somewhat in the direction of darker or lighter shades even in health. The various pigments to which the colour is due will be described in detail further on (chap. ii.). Generally speaking, the depth of colour depends on the quantity of water present; the urine is therefore pale in most cases where the secretion is very abundant, as in chronic Bright's disease, amyloid disease of the kidneys, diabetes mellitus and insipidus, hysteria, and where large quantities of fluid are ingested. The colour is darker where the urine is concentrated, as after abstinence from liquids and where violent perspiration occurs, and it is notably high-coloured in many cases of fever, the so-called 'febrile' urine.

It is convenient, in noting the colour, to compare the urine with objects whose hue is well-known, describing it as straw-coloured, amber, golden, or sherry-coloured. It may happen however, that the urine presents very distinct alterations in colour from the presence of foreign substances, either derived from the body under morbid conditions, or introduced from without in the form of drugs. The following are the most important of these substances :

Blood. The presence of blood may alter the colour of urine considerably, from a bright red to a dull brownish-red. The higher up the urinary tract the source of bleeding is, the more altered is the blood, and the more likely is the latter tint to predominate. Such urines are often described as 'smoky.' If the colouring matter of the blood is present as methaemoglobin the urine is brown rather than red.

Bile. The pigments of bile impart a green tint to the urine, the intensity varying from yellowish-green to greenish-brown, according to the amount of bile present ; on shaking such urine the froth is always tinged yellow. Sometimes the urine itself is not green, but golden in hue, where the pigment of bile is scanty.

Haematoporphyrin. This body, which is iron-free haematin, occurs in very small traces as a normal pigment of the urine ; it is often much increased in sulphonal poisoning, and causes the urine to be of a distinct red or port-wine tint.

Urobilin. This also occurs as a pigment in health in small traces, but is frequently present in larger amount in pathological conditions, such as fever, and where blood-disintegration goes on, as in Addison's disease, pernicious

anaemia, and intracranial haemorrhages. Urine rich in urobilin is often of a deep brown-red tint.

Indigogens or indoxyl-sulphates. These bodies may not affect the colour of the urine as it is passed, though being present as chromogens, *i.e.* substances which can yield colouring matters by oxidation or otherwise; but after the specimen has been allowed to stand for some time it may assume a dirty blue tint from the formation of indigo-blue. This is seen sometimes in cases of typhus fever, and where there is intestinal obstruction or marked constipation, leading to absorption of much indol from the bowel. According to v. Jaksch¹ such urines may occasionally be of a deep yellow tint from the presence of the higher oxidation-products of indol.

Melanin. This pigment also exists as a chromogen—melanogen—when the urine is freshly passed, but on standing it darkens considerably, and may even become black. This may occur in various wasting diseases, as well as in melanotic cancer and sarcoma.

Alcapton. This complex but harmless substance causes the urine, after exposure to the air for some time, to assume a dark colour. It may occur in the urine in health, as well as in phthisis and certain other morbid conditions.

Pus and chyle. Urines containing pus often have a whitish-yellow hue, while in chyluria the colour is milky, owing to the presence of fat and albumin. All the colouring agents just described are derived from the body; the following substances introduced from without also affect the colour of the urine:

¹ *Clinical Diagnosis*, 4th ed., 1897, p. 336.

Carbolic acid and other bodies of the aromatic group. These produce the condition termed carboluria, where the urine is of a greenish-black hue; on shaking, however, the froth is generally white.

Chrysophanic acid, rhubarb, and senna ingested by the mouth, may make the urine orange-coloured or brown. They may, however, produce no alteration in tint till an alkali is added, when the urine assumes a distinct brown-red colour.

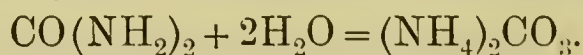
Santonin may cause the urine to become yellow or yellowish-green.

Methylene-blue, which has been employed for neuralgia, malaria, and other diseases, may impart a somewhat startling blue-green colour to the urine; while *fuchsin*, used sometimes in cases of albuminuria, may give it a bright pink tint. When *haematoxylin* has been administered, the urine, on becoming alkaline, may assume a pink colour. If an acid urine does not exhibit this, the addition of ammonia will bring it out.

Although the urine is normally *transparent* it may, without any real change in colour, be more or less *translucent*, especially where much albumin is present. Bacteria in urine also cause it to lose its transparency.

(b) ODOUR. It is not easy to describe this exactly, but it is quite characteristic and is usually said to be "aromatic" in health. It may alter in various ways.

If urine be allowed to stand in a warm place, it soon undergoes chemical decomposition, chiefly under the action of the *micrococcus ureae*, and the urea takes up water and yields carbonate of ammonium, thus:



This is termed the *ammoniacal decomposition* of urine, and the fluid has now a well-marked disagreeable odour, which is largely due to the ammonia produced. The odour of the urine in diabetes mellitus has sometimes been likened to that of freshly cut hay, while acetone, when present, may confer on it an apple-like smell. Various substances taken by the mouth are excreted, more or less changed, in the urine, and impart a special odour to it. Among these may be mentioned turpentine, which causes a rather pleasant smell, resembling that of sweet violets; asparagus, which causes a disagreeable and penetrating odour; cubebs and copaiba, which produce a not unpleasant aromatic smell; and where, as sometimes happens, there exists a fistula between the bowel and the urinary tract the odour becomes faecal.

(c) SPECIFIC GRAVITY. This is determined by the urinometer, the stem of which is graduated from 1000 (taken as the density of distilled water at 15° C.) to 1060. The ordinary instrument has small divisions, each corresponding to two degrees; but, if great accuracy is desired, the scale should register half-degrees, and two instruments may be used, one registering from 1000 to 1030, and the other from 1030 to 1060. In pouring urine into the test-glass for the purpose of taking the specific gravity, the glass should be held obliquely, and the urine allowed to run gently down the side, to avoid the formation of froth; if any does form, it can be removed by a piece of filter paper. The urinometer is then taken, wiped dry and clean, and gently lowered into the urine and left there till it floats stationary. In reading off the result, the observer must not look down upon the instrument,

but must hold the urine-glass on a level with the eye, carefully noting what line on the scale is cut by the under surface of the fluid, or by the lower edge of the meniscus formed where the stem of the instrument meets the urine. When not in use the urinometer may be conveniently kept floating in a cylinder of water. It sometimes happens that the amount of urine supplied for examination is too small to allow of the instrument floating in it; in such a case we may either dilute the urine to a given degree, multiplying the result obtained in the same ratio, or we may employ specific gravity beads, that which just remains suspended in the fluid giving the desired figure. Even a single bead (of low specific gravity) may be employed, a note being taken of the extent to which the sample must be diluted to enable the bead to remain suspended in it. It must be borne in mind that if the density be taken when the urine is warm the result is too low.

In health the specific gravity varies from 1015 to 1025, but may be higher if the urine happen to be concentrated, as after great perspiration. The urine secreted in the early hours of the morning, during sleep, is more dense than that of any other part of the day. In infants the density is much lower than in adults. As urea is the most abundant solid constituent of urine, variations generally depend on the quantity of that substance present.

Pathologically, the specific gravity is lowered in granular contraction, and amyloid disease of the kidneys, in the abundant limpid secretion often met with in hysteria, and in diabetes insipidus; it may fall as low as 1003,

occasionally even lower. The density is raised notably in diabetes mellitus, on account of the large amount of sugar and urea present, and may be 1045 or higher ; it is also raised in the concentrated urine of fever.

From the specific gravity an approximate calculation of the total solids may be made ; in order to do this, the last two figures of the specific gravity are taken, and multiplied by 2.33 (termed Christison's or Häser's coefficient). The result obtained gives the amount of solids in grammes per 1000 c.cm. of urine.

Ex. Suppose the specific gravity is 1022, $22 \times 2.33 = 51.26$ grms. per 1000 c.cm. or 5.126 per cent. If the patient were passing 1500 c.cm. per diem, this would indicate 76.92 grms. of solids, or 2.71 oz. In health, from 50 to 70 grms. ($1\frac{1}{2} - 2\frac{1}{2}$ oz.) of solids are present in the twenty-four hours' excretion of an adult man. To directly determine the total solids, 15 c.cm. of the urine are placed in a small capsule, the weight of which has been accurately determined, and evaporated as fully as possible on a water-bath, and then placed in a hot-air chamber kept at a temperature of 100° C. till the residue is perfectly dry. The capsule and contents are then removed, and placed in a desiccator containing strong sulphuric acid. When quite cool and dry, the weight is again ascertained, and on deducting the weight of the capsule, we have that of the total solids in a given quantity of urine, viz. 15 c.cm.

(*d*) REACTION. The reaction of urine is determined by the use of blue and red litmus paper, the former becoming red with an acid urine, the latter blue with an alkaline one ; if neither show any change the specimen is neutral. It is sometimes difficult to say whether the paper changes

colour slightly, or whether it is merely darkened in tint by being wet with the urine. In that case it is advisable to wet it with water before use, as then any further change is more readily appreciated. Normally, a sample of the whole day's urine is acid, and indeed the urine passed at any time is generally found to be so. It may happen, however, that, about three hours after a meal, the urine may react neutral, or even alkaline. This is due to the secretion of the acid gastric juice, which liberates bases in the blood, and these passing into the urine lower its acidity, or may even neutralize it altogether.

The acidity of the urine is due to the presence of an acid salt—sodium di-hydrogen phosphate (NaH_2PO_4)—and not to a free acid, as is shown by the fact that it gives no precipitate of sulphur with sodium thiosulphate. The alkalinity seen after a meal is due to the presence of alkaline phosphates, *e.g.* di-sodic hydrogen phosphate (Na_2HPO_4), and tri-sodic phosphate (Na_3PO_4), and, perhaps, also to sodic carbonate (Na_2CO_3), that is to say, is due to fixed alkalis. Urine that is undergoing alkaline decomposition owes its alkalinity to ammonium carbonate, that is, to a volatile alkali. Red litmus paper, which has been turned blue by a fixed alkali, does not lose its colour when dried with gentle heat, while that affected by a volatile alkali like ammonia, is restored to its original colour. It sometimes happens that a urine reacts acid to the one kind of paper and alkaline to the other; it is then said to be amphoteric, and owes this property probably to the presence of both di-sodium hydrogen phosphate and sodic di-hydrogen phosphate in certain

definite proportions. The acidity of urine is *increased* by flesh diet, exercise, proteid metabolism, and by the ingestion of mineral acids such as hydrochloric. It is *diminished* during gastric digestion, and after the consumption of organic salts, such as citrates, tartrates, and lactates, which are oxidized in the body, and excreted as alkaline carbonates. In disease it is lowered in anaemia, chlorosis, and debility generally, and is raised in scorbutus and leucocythaemia.

Determination of the acidity of the urine. We can ascertain the 'degree of acidity' of the urine, but we cannot state the absolute acidity in definite terms, as we are not dealing here with a free acid but with an acid salt which, when treated with an alkali, gives a salt that is called a neutral phosphate, but which has itself an alkaline reaction. If we always use the same method we can say that a given urine is so much more or less acid than it was, say, a week before, but we cannot say more than this. In carrying out the process we measure out 100 c.cm. of urine which are placed in a porcelain dish. A burette is then filled with a standard solution of caustic soda of such strength that 1 c.cm. corresponds to (*i.e.* can exactly neutralize) .0063 grms. of oxalic acid. This solution is made by dissolving 4 grms. of pure solid caustic soda in one litre of distilled water. The solution is allowed to run cautiously into the urine, with constant stirring, till the point is reached where the acid reaction to blue litmus paper is lost. The number of c.cm. of soda solution used is now noted, and from this the 'degree of acidity' in terms of oxalic acid is calculated.

Ex. Suppose 20·6 c.cm. of soda solution have been used. $20 \times \cdot 0063 = \cdot 129$. The acidity of this given specimen, therefore, corresponds to 0·129 grms. of oxalic acid per 100 c.cm. of urine.

(e) CHARACTER OF THE DEPOSIT. 1. *Mucous cloud*. This is frequently observed in healthy urine after it has stood for a few hours. It has the appearance of a semi-transparent, filmy cloud occupying the lower fourth of the urine-glass. The modern view is that it consists in general not of true mucin but of a nucleo-albumin.

2. *Urates*. These very commonly appear as a distinct sediment even in health. The deposit is often of a pink, red, or reddish-yellow tint, owing to the presence of the normal colouring matters of the urine carried down by the precipitate. At other times the deposit is almost white. It consists of the amorphous quadriurates of sodium, potassium, and ammonium, and is readily cleared up on the application of heat.¹ This sediment is sometimes spoken of as the 'lateritious' or 'brick-dust' deposit.

3. *Uric acid*. This may occur as a deposit in its free crystalline state not combined with a base. It is usually scanty, but can be well enough recognized by the naked eye, and has been called the 'cayenne-pepper' deposit, from the reddish-yellow colour the crystals exhibit, and which they owe to the urinary pigments. The nature of the crystal can be readily determined by the microscope.

4. *Phosphates*. The ordinary deposit of phosphates forms a distinct white and somewhat flocculent precipitate,

¹Chemical and microscopical tests for this and other deposits will be found in chapter viii.

and consists mainly of ammonio-magnesium phosphate ($\text{NH}_4\text{MgPO}_4 + 6\text{H}_2\text{O}$) along with which may be tri-calcium and tri-magnesium phosphates and ammonium urate. The reaction of such urines is almost invariably alkaline or neutral. If blood be present the phosphatic deposit is often tinged red; not unfrequently pus is mixed with this sediment. The microscope shows the distinctive crystals of ammonio-magnesium phosphate, often described as 'knife-rest' or 'coffin-lid' crystals.

5. *Blood*. If present in tolerably large quantity this forms a brownish-red deposit, the urine at the same time exhibiting a pink or smoky tint; chemical tests or the microscope will clear up any doubt.

6. *Pus*. This frequently forms a heavy white or whitish-yellow deposit in cases of renal and bladder disease. It resembles the sediment of phosphates, but can be distinguished by the use of the microscope and by various chemical tests.

7. *Calcium oxalate*. This seldom forms a copious deposit, but a certain amount may be thrown down, and often becomes entangled in the mucous cloud, giving it a somewhat glittering appearance. The microscope reveals the typical minute octahedral crystals. Rarer deposits are those of carbonate of calcium, acid urate of sodium, leucin, tyrosin and cystin, which will be spoken of in greater detail under the chemical examination of the urine (see chap. vii.).

(f) The last point we need consider, in treating of the urine as a whole, is the **Quantity**.

In general terms it may be said that a healthy adult man passes 1400–1500 c.cm. (45–50 oz.) per diem, a

woman some 200 c.cm. less. Many men in perfect health, however, never pass more than 1200 c.cm. in twenty-four hours, while others may void 1600-1700. A child of six passes about 350 c.cm., and one of twelve years, 600.

The amount is diminished by violent perspiration, diarrhoea, copious vomiting, abstinence from food, and in acute nephritis and chronic parenchymatous nephritis. Decrease in the excretion is sometimes termed *oliguria*, while complete suppression is known as *anuria*. Increase in the flow (*polyuria*) occurs in cold weather (through contraction of the cutaneous vessels), in copious drinking, where much proteid food is taken, in hysterical attacks, during nervous excitement, in cirrhosis and amyloid disease of the kidneys, in diabetes insipidus and mellitus, and under the action of various drugs such as digitalis, certain salts of potassium, diuretin, etc. As noted above, where the quantity is great the colour tends to be pale and the specific gravity low; in diabetes mellitus, however, although the flow is copious, the specific gravity is usually distinctly raised.

Reagents and apparatus required for urinary analysis. In order that the urine may be subjected to chemical examination, certain reagents and apparatus are requisite. For very thorough and elaborate analysis a large number of the former are needed, but for ordinary clinical testing (reaction, albumin, sugar, blood, bile, and peptones) the following will suffice:

Red and blue litmus paper.

Strong acetic acid (33 per cent.).

Strong nitric acid (specific gravity, 1.4).

Strong nitric acid, containing a little nitrous acid.

Picric acid—saturated solution.

20 per cent. solution of caustic potash.

Liquor ammoniae, B.P.

Tincture of guaiac (freshly prepared).

Ozonic ether.

Cupric sulphate solution (10 per cent.).

Fehling's solution, used for both the qualitative and quantitative tests for sugar, has the following composition :

(1) Dissolve 34.64 grms. pure cupric sulphate in distilled water, and make up the volume to 500 c.cm. with water ;
 (2) dissolve 173 grms. of Rochelle salts in 400 c.cm. water, add to this solution 51.6 grms. sodium hydrate, and again make up the volume with water to 500 c.cm. These two solutions should be kept in separate bottles, well-stoppered and not exposed to the light. When about to use, mix equal volumes of the two fluids. 10 c.cm. of such a mixture is exactly reduced by 0.05 grms. of grape-sugar. The *hypobromite of sodium solution* employed in the quantitative estimation of urea is made up as follows :

Caustic soda (solid),	-	-	-	-	-	100 grms.
Bromine,	-	-	-	-	-	25 c.cm.
Water,	-	-	-	-	-	250 c.cm.

The *solution* used with Esbach's tube to determine the amount of albumin in urine is made by dissolving 10 grms. of picric acid and 20 grms. of citric acid in 800 c.cm. of boiling water ; when cool the volume is made up to one litre with cold water.

The various standard solutions employed in other analyses, as well as special reagents used in qualitative tests, will be described in treating of the substances for

which they are employed. The following apparatus will suffice for most purposes.

One dozen test tubes, 6 in. by $\frac{5}{8}$ in. When filled up one inch such tubes hold about 5 c.cm. of fluid.

Small glass funnel, 3 in. diam. at the mouth.

Schleicher's filter-papers, $12\frac{1}{2}$ cm. (5 in.) in diameter.

Two porcelain evaporating dishes, 4 in. wide and $1\frac{1}{2}$ in. deep.

Retort-stand, with 2 in. and 4 in. rings, and clamp (rubber-faced) for burette.

Spirit lamp or Bunsen burner.

Glass stirring-rods.

Wire gauze, 6 in. square.

Two beakers of 150 c.cm. capacity.

A burette of 50 c.cm. capacity, graduated into cubic centimetres and tenths; one provided with a Schellbach's band is much easier to read.

A pipette marked at 5 c.cm.

A cylindrical glass measure of 100 c.cm. capacity, graduated to half cubic centimetres.

An Esbach's tube for albumin.

Some form of ureameter (Dupré's, Doremus's or Gerrard's).

Table of weights and measures. The metric system, on account of its numerous advantages, is universally employed in scientific work. The student may find the following tables of use :

WEIGHT.

1 Milligramme	(0.001 gm.)	=	0.0154 grain.
1 Centigramme	(0.01 gm.)	=	0.1543 grain.
1 Decigramme	(0.1 gm.)	=	1.5432 grains.
1 Gramme		=	15.432 grains.
1 Decagramme	(10 grms.)	=	154.32 grains.
1 Hectogramme	(100 grms.)	=	3.52 ounces.
1 Kilogramme	(1000 grms.)	=	$\left\{ \begin{array}{l} 35.27 \text{ ounces or} \\ 2.204 \text{ pounds.} \end{array} \right.$
1 grain	=	0.064 grms.	
1 ounce	=	28.349 grms.	
1 pound	=	453.59 grms.	

LENGTH.

1 millimetre (1 mm.)	= 0.039 inch.
1 centimetre (1 cm.)	= 0.393 inch.
1 decimetre (1 dm.)	= 3.937 inches.
1 metre (1 m.)	= 39.37 inches.
1 inch	= 2.539 cm.
1 foot	= 30.479 cm.
1 yard	= 91.438 cm.

CAPACITY.

1 cubic centimetre (1 c.cm.)	= 0.061 cubic inch.
1 litre = 1000 c.cm.	= 35 fluid ounces or $1\frac{3}{4}$ pint.
1 fluid ounce	= 28.396 c.cm.
1 pint	= 20 ounces = 567.9 c.cm.

Grammes per hundred, multiplied by 4.375 gives grains per ounce.
 Fluid ounces multiplied by 28.396 gives cubic centimetres.

In scientific work the Centigrade scale is usually employed in recording temperatures. On it the boiling point is at 100° , and the freezing point at 0° . The ordinary scale used for domestic purposes in this country is the Fahrenheit, where 212° is boiling point and 32° is freezing. To convert Fahrenheit into Centigrade, subtract 32 and multiply by $\frac{5}{9}$, *i.e.* $C = (F - 32) \frac{5}{9}$.

To render Centigrade as Fahrenheit, multiply by $\frac{9}{5}$ and add 32, or $F = \frac{9}{5} C + 32$.

Uric acid,	-	-	-	-	-	-	0.55	grms.
Hippuric acid,	-	-	-	-	-	-	0.40	„
Creatinin,	-	-	-	-	-	-	0.90	„
Pigments and other organic bodies,	-	-	-	-	-	-	10.00	„
Chlorine,	-	-	-	-	-	-	7.50	„
Sulphuric acid,	-	-	-	-	-	-	2.00	„
Phosphoric acid,	-	-	-	-	-	-	3.10	„
Ammonia,	-	-	-	-	-	-	0.77	„
Potassium,	-	-	-	-	-	-	2.50	„
Sodium,	-	-	-	-	-	-	11.00	„
Magnesium,	-	-	-	-	-	-	0.26	„
Calcium,	-	-	-	-	-	-	0.20	„
Iron,	-	-	-	-	-	-	A mere trace.	

Total nitrogen in urine. The importance of urine as a vehicle for the elimination of waste nitrogenous matter will become apparent at once when we recollect that of the 16 grms. of nitrogen excreted daily, 15 grms. find their way out of the system through the kidneys. The urinary nitrogen is distributed thus among the various azotised bodies in that fluid :

Urea,	-	-	-	-	-	-	86	per cent.
Ammonia,	-	-	-	-	-	-	3	„
Creatinin,	-	-	-	-	-	-	3	„
Uric acid,	-	-	-	-	-	-	2	„
Xanthin bases, indol, skatol, hippuric acid, etc.,	-	-	-	-	-	-	6	„
								100 per cent.

In making observations on metabolic processes, the effect of diet, etc., it is frequently necessary to estimate the total nitrogen in the urine, which is done by Kjeldahl's method or some modification thereof. The rationale of this process is that the nitrogen present is first of all converted into ammonia; the latter is then liberated by the addition of a fixed alkali, distilled off and received

into an acid solution of known strength. After all the ammonia has been absorbed, the strength of the acid solution is again ascertained, and is found to be less than at first, the difference being due to the ammonia which has entered it. In this way the amount of ammonia, and so the quantity of nitrogen from which it was formed, can readily be calculated.

The details of the process are as follows: Take 5 c.cm. of urine and place in a long-necked flask of 200 c.cm. capacity along with 20 c.cm. of pure strong sulphuric acid, and a small globule of mercury or piece of platinum foil to obviate bumping when the mixture is boiled. Now heat gently on wire gauze till the contents boil, and maintain at this point for thirty minutes; at the end of this time all the nitrogen will be converted into ammonia, which will have united with the acid present.

The liquid must now be allowed to cool and water then added till it measures 100 c.cm. Heat will be developed on the addition of the water, and, when the fluid is again cool, it must be transferred to a larger long-necked flask of 700 c.cm. capacity, solution of caustic soda added till the reaction is alkaline, and the flask shaken several times. The caustic soda is added for the liberation of the volatile ammonia which must now be distilled off. Some recommend that a little, say 10 c.cm. of a strong solution of potassium sulphide, be added to hasten the process. The neck of the large flask must be connected at once with one end of a condenser, the other end of which dips into a standard solution of an acid, say 50 c.cm. of a deci-normal solution of sulphuric

acid. Heat is applied to the flask and the contents brought to the boiling-point and kept there for three-quarters of an hour (fig. 1).

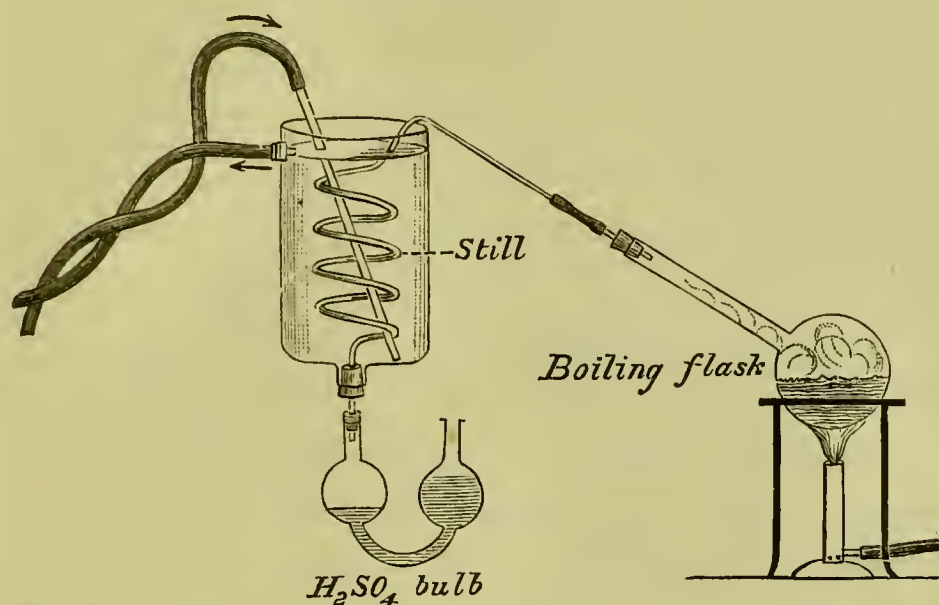


FIG. 1.—APPARATUS FOR KJELDAHL'S METHOD. (Halliburton.)

The receiving flask containing the acid is then removed, and, *after* this has been done, the flame is turned down. It is now necessary to find out how much free acid is in the flask as compared with the amount present at first; the difference will correspond to the amount of ammonia that has entered it. For this purpose we titrate the acid with a deci-normal solution of caustic potash.¹

¹It is appropriate at this stage to explain the meaning of the terms normal and deci-normal as applied to chemical solutions. A normal solution of a body is one containing the molecular weight of grammes of that substance dissolved in one litre (1000 c.cm. of water), provided always that the molecule contains, or corresponds to, only one atom of replaceable hydrogen or metal. If it contain or correspond to two atoms of replaceable hydrogen

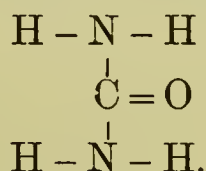
To carry out this process we fill a burette by means of a small funnel with an $\frac{N}{10}$ solution of caustic potash, allow a little to run out to expel all air from the nozzle, and then read off at what level exactly the solution stands. The burette is now fixed in the clamp of the retort stand, and the solution to be titrated is poured carefully into a porcelain dish, care being taken to wash out the flask well with distilled water and to add the rinsings to the fluid in the dish, that no acid be left behind. In order to indicate when sufficient alkali has been added to neutralize all the acid, a little methyl-orange is added to the fluid before we begin; this indicator turns pink in an alkaline or neutral solution. Now run in the solution of caustic potash, with constant stirring, till a permanent pink colour appears, indicating that we have neutralized all the acid. The level of the solution in the burette must now be read off.

or metal, only half the molecular weight is taken; if to four, only one-quarter. *Example.*—A normal solution of caustic potash (KOH) which contains one atom of replaceable metal K, in the molecule, will be the molecular weight or 56 grms., dissolved in one litre of water, and every c.cm. of such a solution will contain 0.056 grms. of caustic potash. A normal solution of sulphuric acid will not be a solution of 98 grms. (its molecular weight), but of half that, or 49 grms. in one litre, as the molecule (H_2SO_4) contains two atoms of replaceable hydrogen, and each c.cm. of such solution will contain 0.049 grms. of sulphuric acid and will be exactly neutralized by 1 c.cm. (=0.056 grms.) of a normal solution of caustic potash, or by the same amount of a normal solution of ammonia (=17 grms. to the litre or 0.017 grms. in each c.cm.). A deci-normal solution is one of one-tenth the strength of a normal solution, and is often indicated by the formula $\frac{N}{10}$.

Ex. Suppose 20 c.cm. of the alkaline solution have been used ; this indicates that 20 c.cm. of the acid solution are left in the dish. But 50 c.cm. were put at first into the receiving flask, so that the difference or 30 c.cm. must have been neutralized by the ammonia obtained from the nitrogen in the urine. 30 c.cm. of deci-normal acid = 3 c.cm. normal acid = 3 c.cm. normal ammonia ; each c.cm. of this latter contains 0.017 grms. of which 0.014 grms. are nitrogen. 3 c.cm. therefore contain 0.042 grms. nitrogen and this was obtained from 5 c.cm. urine. If the patient passed 1550 c.cm. of urine per diem, the total urinary nitrogen would be

$$\frac{1550 \times 0.042}{5} = 13.02 \text{ grms.}$$

Urea. This, the most important constituent of urine, has the general formula $\text{CO}(\text{NH}_2)_2$ and the constitutional one



It is isomeric with cyanate of ammonium, from which it was first prepared by Wöhler in 1828. As already mentioned, it is the form in which about 86 per cent of the nitrogen of the urine is combined, and is therefore the chief means by which that element is eliminated from the system. Its chief but probably not exclusive seat of formation is the liver.

The amount of urea excreted daily varies from 30 to 35 grms., a fair average for a healthy adult being 33 grms. (500 grs.), or 2 per cent. of the whole urine. The latter figure will of course depend largely on the degree of

concentration of the urine, and is often higher. The urea excretion is increased when much flesh food is taken, and, to a much less extent, by muscular exercise; it is also increased in fevers, and largely so in diabetes from the large amount of proteid food ingested. It is diminished in starvation, in acute yellow atrophy of the liver, and in chronic parenchymatous disease of the kidneys.

Tests for urea. (1) *Qualitative.* Urea forms well-marked crystals with nitric acid ($\text{CO}(\text{NH}_2)_2\text{HNO}_3$), and with oxalic acid ($\text{CO}(\text{NH}_2)_2\text{C}_2\text{H}_2\text{O}_4\text{H}_2\text{O}$). If, therefore,

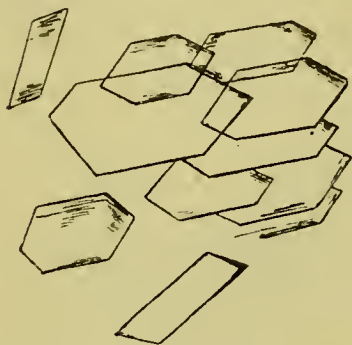


FIG 2.—CRYSTALS OF UREA NITRATE.

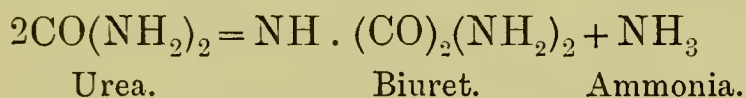
we concentrate a little urine by evaporation, which can be readily accomplished on an extemporized water-bath (made by laying a porcelain dish containing the urine on the top of a tin can in which water is kept boiling), and place a drop of the syrupy liquid on a slide along with a drop of nitric acid, we

get a speedy formation of crystals of urea nitrate of a rhombic or hexagonal form, readily recognizable under the microscope (fig. 2). A little urine and nitric acid mixed may even be evaporated on the slide itself, and the crystals produced in this way. It occasionally even happens that the addition of nitric acid to urine in a test-tube produces a precipitate of urea nitrate.

If it be desired to prepare urea itself, the student should proceed thus: take 100 c.cm. of urine and evaporate to a syrupy consistency in a porcelain dish on the water-bath. Remove the dish to let the contents cool, and then set it in another one containing cold water, and

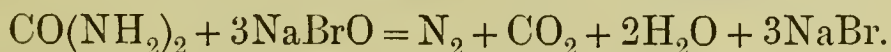
add an excess of strong pure nitric acid. A mass of crystals of urea nitrate is formed, which, after the excess of liquid has been poured off, may be dried between two layers of thick filter-paper. The crystals are then placed in another dish, and carbonate of barium in powder added, which has the effect of depriving the urea nitrate of its acid, forming barium nitrate, and leaving the urea uncombined; plenty of the carbonate should be taken. The whole mixture must now be stirred up with methylated spirits to form a paste, again evaporated to dryness, and then treated with absolute alcohol to dissolve out the urea. Filter, and evaporate a little of the filtrate on a slide, and rhombic crystals of pure urea will separate out.

Urea, which has a saline taste, is soluble in water and in alcohol, insoluble in ether; if heated in a test-tube, it gives off ammonia, and a substance called biuret is left:



If to the biuret a drop of one per cent. solution of cupric sulphate, and a few c.cm. of a twenty per cent. solution of caustic potash be added, a rose-pink colour is developed (biuret reaction).

(2) *Quantitative estimation.* This, to be performed with great exactness, is a matter of some difficulty requiring the resources of a laboratory, but clinically a tolerably accurate result may be got by the hypobromite method (Knof and Hüfner's). The principle on which this rests is that urea when acted upon by an alkaline hypobromite, yields nitrogen, carbonic acid, water, and a bromate, thus:



Hypochlorous acid acts in a similar way.

If then we make use of some form of apparatus in which this decomposition can be effected, and if we collect and measure the nitrogen gas alone, we have a means of estimating urea quantitatively, since 1 grm. of urea contains 0.46 grms. of nitrogen, which occupies a volume of 372 c.cm.

An objection raised against this method is that other bodies in urine, notably uric acid and creatinin, yield nitrogen too; as a set-off to this, however, some of the urea nitrogen does not become free, probably going to form a cyanate, or as some think, a nitrate.

The hypobromite solution is prepared as follows: 100 grms. of sodium hydrate are dissolved in 250 c.cm. of water and to this 25 c.cm. of bromine added; the mixture should be kept in a well-stoppered bottle. As it does not keep well it is better to make smaller quantities at a time, which can be conveniently done as follows:

Small sealed tubes containing 2.2 c.cm. of the bromine can be purchased, each of which will make 25 c.cm. of the hypobromite solution. This is done by pouring 24 c.cm. of solution of caustic soda of the proper strength (which keeps quite well) into a stout bottle provided with a stopper; one tube of bromine is then dropped in, the bottle closed and smartly shaken to break the tube; the liberated bromine at once unites with the soda, and we have about 25 c.cm. of fresh hypobromite solution of a distinctly alkaline reaction. There are various forms of apparatus which may be employed in the estimation, but only two will be described in detail.

(1) *Dupré's apparatus* (fig. 3). This consists of a cylinder, A, about 50 cm. high and 10 cm. in diameter, filled three-

quarters full of water, into which an inverted burette, B, dips, being held in any desired position by means of a clamp. To the exposed end of the burette is fastened a

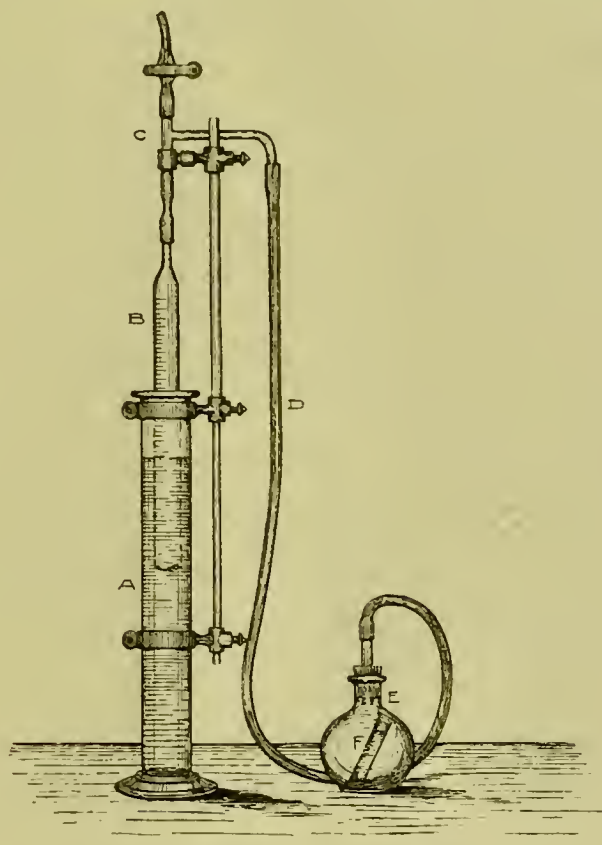


FIG. 3.—DUPRE'S UREA APPARATUS.

small \perp -shaped glass tube, C, having attached to one end a short length of open rubber tubing, which can be closed at will by a clip, while its other aperture communicates, by means of a tube, D, with a conical glass, E, provided with a well-fitting stopper. To use the apparatus, measure out 25 c.cm of the hypobromite solution and pour it into the conical glass. Then carefully measure out 5 c.cm of urine into the small test-tube, F, which accompanies the apparatus, and place it within the jar in a sloping position, so that the two liquids cannot mix. It is necessary that the urine be first freed from albumin,

if any be present; for this purpose 20 c.cm. of urine may be brought to the boil in a small beaker, treated with a few drops of acetic acid, and boiled for two minutes more. The albumin is precipitated and the urine may now be filtered. The filtrate will probably be under 20 c.cm., so that cold distilled water must be added to it to make it up to this quantity; of this, 5 c.cm. may be used for the analysis.

Having laid the small tube containing the urine within the jar, which can be easily done by inserting the tip of the finger into the mouth of the tube, the jar should be closed tightly. It is now necessary to have the burette at such a level in the water that the line marked 0 c.cm. is on a level with the water in the cylinder, and the water in the burette at the same level too; this is accomplished by loosening the clip, raising or lowering the burette as required, and then tightening the clip again. The conical glass containing the hypobromite is now tilted to such an angle that the urine flows out of the little tube; a brisk development of gas takes place, with the evolution of heat, and the water in the burette is displaced by the nitrogen that comes off; the carbonic acid produced is all absorbed by the excess of alkali in the hypobromite solution. The conical glass should be shaken gently several times to ensure thorough mixing, and then set in a bowl of water of the same temperature as that in the cylinder. At the end of ten minutes, the burette is raised till the level of the water in it corresponds to that in the cylinder, and the number of c.cm. of nitrogen gas obtained, read off.

Calculation. 1 grm. of urea should yield 372 c.cm. of

nitrogen, but in actual practice some is lost, so that really 35.4 c.cm. are got from 1 gram. of urea, or 35.4 c.cm. from 0.1 gram.

Ex. Suppose 32 c.cm. of gas are obtained in an analysis, then:

$$35.4 : 32 :: 0.1 : x, \text{ and } x = 0.09 \text{ grms.}$$

This amount of urea was present in 5 c.cm. of urine; if the patient were passing 1470 c.cm. per diem, the total urea would be:

$$\frac{1470 \times 0.09}{5} = 26.46 \text{ grms.}$$

The percentage is easily got by multiplying the amount of urea in 5 c.cm. by twenty, in this case it would be 1.8.

If we wish for greater accuracy, the volume of gas given off should be corrected for temperature and pressure, 35.4 grms. of nitrogen being obtained from 0.1 gram. urea really at 0° C. (=273° absolute temperature) and 760 mm. pressure of the barometer. Suppose the room be at a temperature of 16° C., and the barometer register 740 mm. pressure; the real volume of gas obtained in our analysis would be:

$$32 \times \frac{740}{760} \times \frac{273}{273 + 16} = 29.7 \text{ c.cm.}$$

of nitrogen from 0.1 gram. urea, and the rest of the calculation would be altered accordingly. As stated above, urea does not give off all the nitrogen it should; diabetic urine, however, yields nearly the full amount, *i.e.*, about 37.0 c.cm. for 0.1 gram. urea.

(2) *Doremus' ureameter.* This simple and cheap apparatus consists of a glass bulb with a short neck and open mouth, having attached to it, at the other side from the

aperture, a longer closed limb, set at an angle of about 40° . This limb is the collecting chamber for the gas, and is graduated in a certain scale (fig. 4). Accompanying this

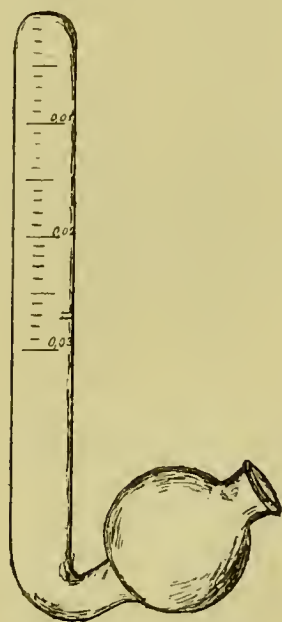


FIG. 4.—DOREMUS' UREAMETER.

is a small 1 c.cm. pipette, provided with a rubber nipple. A little urine, free of albumin, is poured into a small beaker, and enough drawn up into the pipette to reach the graduating mark. This can be easily accomplished, after a little practice, by gently squeezing the nipple, and then dipping the end of the pipette into the urine, when, on relaxing the pressure, the liquid is sucked up into the tube. It will probably be found (as is intended) that it does not reach the 1 c.cm. mark. Still keeping the nozzle submerged in the urine, again compress

the nipple, and expel the urine from the tube, and a few air-bells after it, and then once more relax the pressure and allow the urine to flow up towards the mark. After one or two trials the exact quantity will be obtained. The pipette may now be left ready charged in the beaker, while the ureameter is being prepared. Holding the instrument erect, pour about 12 c.cm. of hypobromite solution into the bulb, and then, by inclining the closed limb downwards, allow it all to flow into the latter; if the proper quantity be taken, the tube will be filled up to the point where it is marked by two parallel lines. Now, hold the instrument, still with the closed limb downwards and the open mouth of the bulb facing the operator, and blow in from a wash-bottle sufficient water

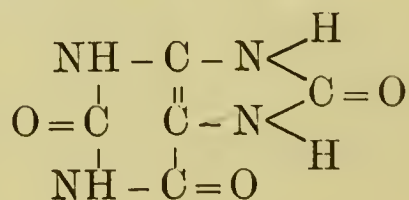
to fill up the long limb and half the bulb. Still keeping the instrument in this position, take the charged pipette, wipe its end quickly, and then, rapidly inverting the ureameter till the long limb is almost vertical, promptly slip the pipette into the bulb until its turned-up nozzle is projecting through the bend into the closed limb. The moment it is there, begin gently to express the urine, and a brisk evolution of gas will take place in the collecting tube. If the gas threaten to pass out of the latter into the bulb, refrain from pressure for a few seconds, and then recommence and go on till all the urine has been expressed; the latter being much lighter than the mixture of hypobromite and water, usually ascends through it with little delay. The instrument may now be set aside for fifteen minutes, and then the reading taken. The graduations on the limb represent centigrammes of urea and tenths; by multiplying by a hundred we at once get the percentage. Suppose, for example, the fluid stands at the level of 0.023, this indicates the amount of urea in grms. obtained from 1 c.cm. of urine, and the percentage would be 2.3, from which the daily total could be quickly computed.

(3) *Gerrard's ureameter* is worked on the same principle as Dupré's, but in this instrument the burette is larger in calibre and more like a cylinder. By its lower end it communicates, by means of a rubber-tube, with a glass reservoir which is clamped to it, and can be moved up and down on the cylinder. The latter has at its upper end a T-shaped tube, one end of which is closed by a clip and can be utilized to admit air when required, while the other end communicates with a conical glass con-

taining the urine and hypobromite. The two latter substances having been placed in the vessel which holds them, but not mixed as yet, and the stopper inserted, the clip is opened, and the reservoir filled with water, and raised till the latter flows into the measuring cylinder up to the line marked 0. When this is done, it should be seen that very little water remains in the reservoir. The clip is now closed, the urine and hypobromite mixed, and the gas, coming off, displaces the water in the cylinder, driving it back into the reservoir. Finally, the latter is pushed down till the water in it and the cylinder are on the same level, and the figure on the latter read off; it gives parts of urea per hundred.

The mercuric nitrate, or Liebig's method of estimating urea is more troublesome, and has become so generally replaced by the easier hypobromite process that it is little used now. It is based on the fact that urea forms an insoluble white compound with mercuric nitrate, having the formula $(\text{CO}(\text{NH}_2)_2)_2\text{Hg}(\text{NO}_3)_2(\text{HgO})_2$. By using a standard solution of mercuric nitrate, which we know will combine with a definite amount of urea, and an indicator to determine when the end reaction has been reached, we can find out how much urea there is in a given quantity of urine. This method will not be described at greater length as it is daily becoming more obsolete.

Uric acid and the xanthin bases. Uric acid ($\text{C}_5\text{H}_4\text{N}_4\text{O}_3$) is always present in the urine, even in starvation, and possesses the constitutional formula

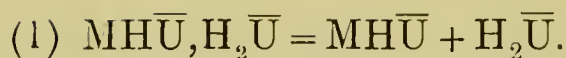


It does not occur free in urine but combined with sodium potassium, and ammonium. Uric acid is dibasic and forms both neutral and acid salts, *e.g.* sodium urate $C_5H_2Na_2N_4O_3$ and sodium biurate $C_5H_3NaN_4O_3$. The neutral salt however never occurs either physiologically or pathologically, but only as a laboratory product, being extremely unstable. The biurates occur in gouty deposits pathologically. The observations and experiments of Roberts¹ have established the fact that the ordinary uratic deposit consists of a third variety of salt, the quadriurate, having the general formula, $MH\bar{U}, H_2\bar{U}$, where M represents a monad metal and \bar{U} the acid radical of uric acid ($C_5H_2N_4O_3$). Sodium quadriurate would thus be $NaH\bar{U}, H_2\bar{U}$. This compound, under the influence of water, breaks up into the biurate and uric acid, and this double salt is also the form in which uric acid is excreted in serpents and birds.

If a little uratic deposit be collected on a filter-paper, washed with absolute alcohol and then dried, and a speck of the dried deposit mixed with plenty of distilled water on a glass slide, the microscope will enable one to see the small amorphous granules undergoing the decomposition mentioned above. After a short while, rhombic crystals of uric acid appear all over the field, the biurate that is formed going into solution.

The same thing takes place, though more slowly, in urine aided by the influence of the acid phosphates. If a little urine be placed in a test-tube (with a drop or two of chloroform to prevent decomposition) and the tube corked, a deposit of uric acid crystals will be found in a day or two. The following equations represent what takes place:

¹ *Uric Acid, Gravel, and Gout*, 1892.



The uric acid is precipitated and the biurate is now acted on by the acid phosphates, giving us the quadriurate once more :

(2) $2\text{MH}\bar{\text{U}} + \text{MH}_2\text{PO}_4 = \text{MH}\bar{\text{U}}, \text{H}_2\bar{\text{U}} + \text{M}_2\text{HPO}_4$, and this goes on till all the uric acid is precipitated. In gout, Roberts believes that the quadriurates of the blood (which are in excess) take up alkali and so produce the biurate which becomes deposited as chalk-stones, etc. He also gives reasons for holding that the factors which influence the solution or precipitation of uric acid in the urine are (1) the amount of uric acid present (2) the degree of acidity of the urine (3) the amount of pigment and (4) the amount of neutral salt. If, in urine, the former two are high and the latter two low, we get a precipitate of uric acid, and this lies at the root of the pathology of uric acid gravel. In birds and serpents uric acid is the form in which waste nitrogen is mainly excreted and corresponds to urea in mammals ; it is almost entirely produced in the liver. In mammals it is possible, even probable, that uric acid arises in quite another way, viz. from the metabolism of the leucocytes and other tissues rich in nuclein. This view, which was suggested by Horbaczewski,¹ is supported by many facts.

The amount of uric acid excreted daily varies from 0·5—0·75 grms. (7-10 grs.). It varies with the nature of the food to a much less extent than urea, but is generally increased under a proteid diet. The amount depends also on the age of the person, being relatively much increased in the newly-born ; exercise increases it to a slight extent, while rest diminishes it, and it is

¹ *Zur Theorie der Harnsäurebildung*, etc., 1892.

increased by certain drugs such as pilocarpine and salicylic acid, and diminished by atropine. Pathologically, it is greatly increased in most cases of leucocythaemia, presumably being derived from the nuclein of the greatly increased number of leucocytes, and may attain several grammes; it also rises in fevers, during thyroid medication, and frequently in cancer, where there is great leucocytosis. It is diminished in chronic nephritis and in gout. A copious deposit of urates gives no indication that the total excretion is above the average.

Uric acid can be readily obtained from urine by adding to it one-twentieth of its volume of strong hydrochloric acid and leaving it to stand for 24 hours. A deposit of deeply pigmented uric acid crystals can then be collected.

Tests for uric acid. (1)

Qualitative.

(a) The microscope is always of value here when the crystals are present, as their rhombic form is very characteristic. Sometimes they are shaped like whet-stones, and in other cases they are barrel-shaped (fig. 5).

(b) The murexide test. This depends on the production of a purple colour—whence its name (Lat. *murex*, a snail, from which the Romans obtained a purple dye). To perform it, a little uric acid or deposit of urates is evaporated by gentle heat in a porcelain dish along with a few drops of moderately strong nitric acid.

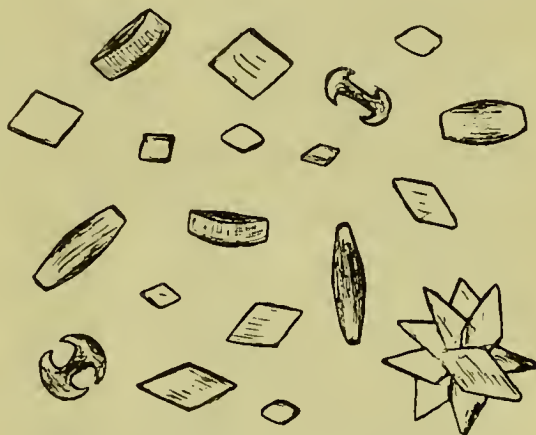


FIG. 5.—CRYSTALS OF URIC ACID.
Various forms.

Decomposition takes place and eventually a yellowish-red deposit is left. When this is cool, a drop or two of ammonia is applied by a glass rod, whereupon a fine purple-red colour develops, which becomes blue on the further addition of caustic potash. This purple colour is due to purpurate of ammonium. If caustic potash solution be used at first instead of ammonia, a violet tint results.

(c) Uric acid readily dissolves in a weak solution of alkali, and this solution, if boiled with Fehling's reagent, reduces the latter.

(2) *Quantitative estimation.* While Salkowski's method is that used in the laboratory, the most accurate process for clinical work is that introduced by Gowland Hopkins.¹ It depends upon the ease and thoroughness with which the uric acid can be precipitated by ammonium chloride. To perform it, measure out 100 c.cm. of urine into a beaker, and add to it powdered neutral ammonium chloride till the urine is quite saturated and a trace of the salt remains undissolved. Usually about one ounce of the salt, more or less, is required. The urine must be well stirred from time to time, and it is best to allow it to stand for at least an hour. On filtering now, the paper will retain a reddish-yellow deposit of biurate of ammonium with a trace of white ammonium chloride. This deposit is washed on the filter-paper two or three times with a saturated solution of ammonium sulphate to remove, as fully as possible, the traces of ammonium chloride. A little water should now be boiled in a wash-bottle and then, having carefully lifted the paper

¹ *Journ. of Path. and Bact.*, vol. i., p. 451, 1893.

from the funnel and opened it out, we wash the deposit completely, with a jet of hot water, into a porcelain dish. A pinch or two of sodium carbonate is added to the hot mixture, and, on stirring, the deposit goes into solution. One or other of two courses may now be pursued. If a chemical balance and other resources of a laboratory be at hand, the alkaline solution is treated with strong HCl which precipitates the uric acid; the precipitate is collected on a filter-paper of known weight, dried and weighed, whereby we know the weight of the uric acid in 100 c.cm. of urine.

Usually, however, in clinical work, the following course is adopted. The alkaline solution is treated, when cool, with 20 c.cm. of pure strong sulphuric acid, which leads to the evolution of a good deal of heat. A burette is filled with a one-twentieth normal solution of potassium permanganate (1.578 grms. to the litre), and this is run into the hot acid liquid till a pink tint, remaining permanent for a few seconds, is attained. This pink colour disappears on standing, and the observer must not be misled thereby. The principle of this method is that uric acid is oxidized by the permanganate to a colourless compound. We now read off the number of c.cm. used, and, as each corresponds to 0.00375 grms. of uric acid, it is easy to calculate the total quantity present.

Ex. Suppose 17.9 c.cm. of permanganate solution are required in an analysis: $17.9 \times .00375 = 0.067$ grms. of uric acid in 100 c.cm. of urine, *i.e.* .067 per cent. If the patient passed 1240 c.cm. urine in twenty-four hours, the total would be $.067 \times 12.4 = .834$ grms. of uric acid.

The xanthin bases. Allied in chemical constitution to uric acid are certain substances in urine collectively described as the xanthin bases, because, in the first place, they are derivatives of xanthin ($C_5H_4N_4O_2$), which contains one atom less of oxygen than uric acid, and because, secondly, they are basic in character. They are sometimes called the 'alloxur' bases, and they and uric acid together may be called the alloxuric bodies, because they all are allied chemically to alloxan, an oxidation product of uric acid. As examples may be cited hypoxanthin ($C_5H_4N_4O$), guanin ($C_5H_5N_5O$), and adenin ($C_5H_5N_5$). They generally amount to one-tenth the quantity of uric acid, and, like it, are increased where there is great destruction of nuclein-containing tissue and in leucocythaemia.

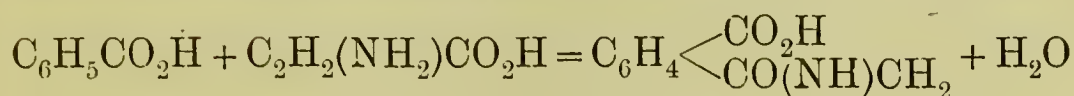
Tests. They exhibit Weidal's reaction, which resembles the murexide test for uric acid, except that chlorine water is used instead of nitric acid.

The quantitative estimation is too elaborate for clinical work, and is of no real importance in ordinary practice. It is performed, according to Salkowski's¹ latest method, by precipitating the uric acid and xanthin bases together from a large quantity of urine by means of a solution of silver and magnesia; the bases are then dissolved out, and filtered off from the uric acid, precipitated again, and estimated as a silver-xanthin compound.

Hippuric acid ($C_9H_9NO_3$) exists in human urine to a small extent as a hippurate. Strictly speaking, it should be considered with the aromatic substances, as it contains, like phenols and cresols, the benzene nucleus. In its chemical

¹ Pflüger's *Archiv.* lxi., s. 268-306, 1898.

nature it is a compound of benzoic acid ($\text{C}_6\text{H}_5\text{CO}_2\text{H}$) and glycocine or amidoacetic acid ($\text{C}_2\text{H}_2(\text{NH}_2)\text{CO}_2\text{H}$), thus :



This synthesis takes place in the kidneys, as has been proved experimentally. The ingestion of any kind of food containing aromatic bodies, which will yield benzoic acid, will increase the output of hippuric acid, and accordingly we find it more abundant after eating plums, pears, cranberries, and mulberries. It is also more plentiful in the urine after a person has been taking benzoic acid by the mouth. In man, the amount excreted daily is from 0.4 grms. (6 grs.) upwards. It is very abundant in the urine of herbivora, and may be easily obtained from this by adding milk of lime to the fresh urine, when calcium hippurate is formed. The urine is then filtered, the filtrate concentrated by evaporation, and strong hydrochloric acid added, when hippuric acid is precipitated as needles or rhombic prisms. The crystals resemble those of uric acid and ammonio-magnesium phosphate slightly, but can be distinguished from the former by not giving the murexide reaction, and from the latter by not dissolving in acetic acid.

Hippuric acid is of little importance from the point of view of clinical diagnosis.

Creatinin ($\text{C}_4\text{H}_7\text{N}_3\text{O}$) is another of the nitrogenous constituents of urine, and is chemically of the nature of a base. It is probably derived from the creatin ($\text{C}_4\text{H}_9\text{N}_3\text{O}_2$) of muscle ingested in our food. Johnson, however, has shown that though the creatinin in urine is isomeric with, and very similar to, that derived from

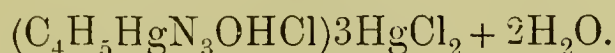
creatin artificially, it differs from it in certain points. Creatinin is of no great importance clinically, except that it has the power of reducing Fehling's solution, and so may lead to error in testing for glucose. The amount excreted daily is about 1 grm., and it depends chiefly on the amount of creatin taken in our food. It is increased in acute illnesses attended with fever, and in diabetes mellitus; diminished in anaemia, chlorosis, inanition, and progressive muscular atrophy.

Tests. (1) *Weyl's reaction.* Pour into a test-tube 5 c.cm. of urine, which will fill an ordinary tube to the extent of about one inch from the bottom. Add five drops of a dilute freshly-made solution of sodium nitroprusside, and then 2 c.cm. (30 drops) of a 10 per cent. solution of caustic soda. A fine ruby-red colour appears, which soon passes into an amber yellow. If the solution be now boiled and a little acetic acid added a fine green colour is developed.

(2) *Jaffé's test.* To 5 c.cm. of the urine in a test-tube, add ten drops of a saturated solution of picric acid, and then 2 c.cm. of solution of caustic potash. A deep red colour results.

(3) Creatinin has the property of forming definite crystalline compounds with salts of some of the heavy metals, notably with zinc chloride and mercuric chloride. The former of these forms rosettes of needles, having the formula $C_4H_7N_3OZCl_2$; the latter also forms a crystalline compound, and by this means we can roughly estimate creatinin quantitatively, proceeding as follows: To 50 c.cm. of urine add 2.5 c.cm. of a saturated solution of sodium acetate, and then 12.5 c.cm. of a saturated solution of

mercuric chloride. A precipitate of sulphates, phosphates, and urates at once forms and must be removed by filtration. The filtrate contains the creatinin as yet in solution, but, after twenty-four hours' standing, it is precipitated in the form of a mercury compound of the formula



The microscope shows it to be composed of a vast number of tiny crystalline spheres. For quantitative estimation Halliburton¹ suggests that the precipitate should be collected on a filter paper of known weight, dried and weighed; one-fifth part of the weight of the compound is creatinin.

The pigments of healthy urine. These are four in number, viz. (a) urochrome, the essential colouring matter of the urine; (b) uroerythrin, the colouring matter of pink urates; (c) urobilin, and (d) haematoporphyrin. The two latter are present in traces only in health, and often as chromogens, so that further description of them will be reserved for the chapter dealing with pathological pigments.

Urochrome long baffled observers, because it possesses no absorption band, and, therefore, could not be recognized by the spectroscope. Garrod² and other observers, however, have devised means of separating it from the urine, when it may be obtained as a brown amorphous powder, readily soluble in water and alcohol, insoluble in ether. There is no doubt that it and not urobilin as formerly supposed, is the body to which healthy

¹ *Ess. of Chem. Physiol.*, 1899, p. 119.

² *Proc. Roy. Soc. Lond.*, vol. lv., 1894, p. 394.

urine owes its colour, but it is quite possible that it is related chemically to urobilin.

Uroerythrin, which colours deposits of urates pinkish-red, is an unstable pigment which can be extracted from a deposit of pink urates by collecting them on a filter-paper, washing with very cold water, and then drying in a warm chamber. The precipitate should then be soaked for some hours in absolute alcohol, the latter poured off, and the residue dissolved in warm water. If the solution be now gently shaken up with amyl alcohol, the latter will extract the pigment, and appear of a rich orange colour. It is apparently much influenced by functional and organic affections of the liver, and possesses a complex absorption band.

Indigogens or indigo-yielding bodies. These may be conveniently described at this point, being connected on the one hand with the pigments, and on the other with the aromatic substances which we shall next consider.

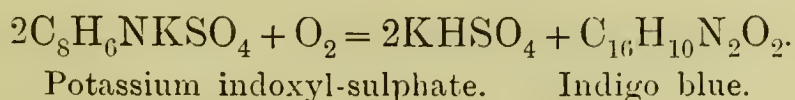
Indol (C_8H_7N) and *skatol* or *methyl-indol* (C_9H_9N), which are constituents of faeces, are always absorbed to a certain extent, and excreted in the urine as indoxyl and skatoxyl-sulphuric acid combined with potassium. The former compound occurs in much the greater quantity, and is sometimes termed indican, as it yields indigo on oxidation; it is, however, different from the indican of plants, which is a glucoside. The formula of indoxyl-sulphuric acid is $C_8H_7NSO_4$, and that of the potassium salt is $C_8H_6NKSO_4$. It is not abundant in human urine, but plentiful in that of the horse. It may be regarded as a chromogen, since it can yield a colour by oxidation. This sometimes, though rarely, occurs spontaneously, and

the urine assumes a bluish tint. As indol is a product of albuminous decomposition, anything that leads to this tends to increase the indoxyl-compounds in the urine. Sahli states that it is increased in constipation, cancer of the liver and stomach, putrid abscesses, peritonitis, gangrenous processes, and starvation.

It may frequently be observed, on adding nitric acid to urine when testing for albumin, that a coloured ring is developed at the junction of the fluids, often of a reddish-violet tint; this is due to the action of the acid on the indigogens, and on another pigment to be described presently called urorosein.

Tests for indigogens. In performing these it is first of all necessary that albumin, if present, be removed from the urine; this may be accomplished in the manner already described (see p. 28). It is also important to make sure that the urine does not contain any salts of iodine.

(1) *Jaffé's test.* Pour 5 c.cm. of urine into a test-tube, and add an equal volume of strong hydrochloric acid, for the purpose of breaking up the potassium indoxyl-sulphate. Then by means of a pipette add cautiously, drop by drop, a solution of bleaching powder of the strength of 1 : 20, when a blue colour will quickly develop. If now a little chloroform be added and the tube shaken gently, the chloroform will dissolve out the indigo-blue which has been formed, and sink with it to the bottom of the tube. The following equation represents what takes place:



If too much of the oxidizing solution be added, the indigo-blue is oxidized to a yellow compound called isatin, and so the blue colour is lost.

(2) *Weber's test*. Place in a test-tube equal quantities of urine and strong hydrochloric acid; add a few drops of dilute nitric acid and boil, when a violet colour is produced. When cool add 2-3 c.cm. of ether, and shake. The ether rises to the top, coloured blue, while the rest of the fluid remains reddish in tint, from indigo-red. Generally speaking, tests carried out at the higher temperature lead to the production of at least some indigo-red.

(3) A third and neat test is performed by placing a crystal of chlorate of potassium at the bottom of a test-tube, adding some urine, and then allowing strong hydrochloric acid to trickle down the side of the tube. The latter will raise the urine up on its surface, and at the junction of the two a delicate purple band will be seen. The chlorate here acts as the oxidizer in the presence of the acid.

Rosenbach's reaction is the name given to the development of a burgundy-red colour in urine (the froth being at the same time bluish-red), when concentrated nitric acid is allowed to fall, drop by drop, into a test-tube of boiling urine. It is due to the formation of indoxyl- and skatoxyl-red.

Urorosein is a red pigment which exists as a chromogen in urine, from which the colour may be produced by the addition of a strong mineral acid such as hydrochloric. The red colour seen occasionally on the addition of a strong acid to urine is usually a mixture of urorosein

and indigo-red. Urorosein is soluble in amylic alcohol, is destroyed by alkalies, and is insoluble in ether, in this latter point differing from indigo-red, which is soluble in that menstruum. Urorosein has a distinct absorption band in the green part of the spectrum. Its chromogen, which has been obtained by Rosin from the urine of the ox, is said to be often met with in the urine of chlorotic patients.

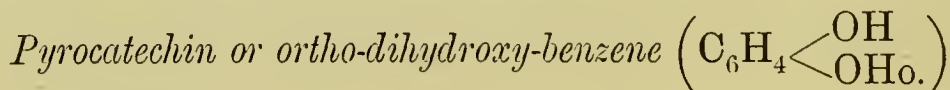
Aromatic compounds in urine. These bodies are so named because, like many other substances of a similar kind, they are chemical derivatives of the benzene molecule C_6H_6 . Many of them are also members of the class of substances in urine called *conjugated sulphates*, because they are excreted conjugated with, or joined to, sulphuric acid. We shall see later on when treating of this acid that one-tenth of that present in urine is in the form of this organic combination (sometimes termed ethereal sulphuric acid). These bodies are derived partly from the food, and partly arise from albuminous decomposition in the bowel. The indigogens which we have already discussed are also conjugated sulphates, and the substances we are now considering are, like them, increased in great constipation and in intestinal obstruction.

As examples of them may be mentioned phenol or hydroxy-benzol (C_6H_5OH), and cresol or hydroxy-toluol (C_7H_7OH), which occurs chiefly as para-cresol; of these two bodies traces are normally present, especially of the latter.

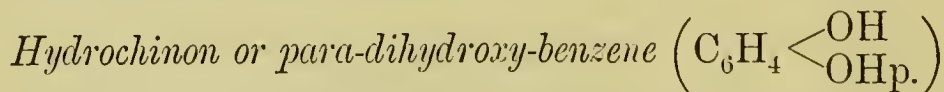
Tests. They may be demonstrated either in the sulphuric acid or in the phenol part of the compound.

(1) To show that they are conjugated sulphates take 5 c.cm. of urine in a test-tube, and add an equal volume of baryta-mixture;¹ a precipitate forms due to the inorganic sulphates being thrown down. The liquid must now be filtered, and to 5 c.cm. of the clear filtrate excess of hydrochloric acid added, and the whole boiled. The turbidity which appears is due to the sulphuric acid which was previously in organic combination, and which has become liberated by boiling with hydrochloric acid.

(2) The phenol part may be detected by heating 200 c.cm. of urine in a distillation flask along with enough dilute sulphuric acid to make it strongly acid. The conjugated compound is broken up and the distillate consists, in part at least, of phenol. If to it we add a little bromine-water, we get a whitish-yellow precipitate of tri-bromophenol. If part of the distillate be heated with Millon's reagent (acid nitrate of mercury) a red colour develops.



also exists in healthy urine in minute traces. To demonstrate it, when sufficient is present to permit of our doing so, we must acidify the urine and shake it up with ether, which extracts the pyrocatechin; if to the ethereal extract a little very dilute solution of ferric chloride be added, a violet colour is the result.



is chemically closely allied to pyrocatechin, but does not

¹ Made by mixing one volume of barium nitrate solution with two of barium hydrate solution, both saturated in the cold.

seem to occur normally in urine; it is found in cases of carbolic acid poisoning.

All these organic sulphuric acids occur, when present, as potassium salts.

In addition to these bodies, traces of other aromatic compounds occur in urine, chiefly derived from the decomposition of albuminous material in the intestine, *e.g.* para-hydroxy-phenyl-acetic acid, hydro-para-cumaric acid, and homogentisic acid. All compounds of this class are increased in intestinal obstruction and constipation, in carbolic acid poisoning, during the administration of drugs of the phenyl class, and in the condition called alcaptonuria.

Other rarer organic bodies in urine. A minute trace of a *nucleo-albumin* is frequently present, and, as already stated, the mucous cloud which forms when urine stands, is probably due to this body. Tests applicable to it, and distinguishing it from mucin, will be described when dealing with the proteids in pathological urine.

Oxalic acid ($C_2H_2O_4$). This is present normally in urine to a slight extent—10 to 15 mgrs. per diem. It may be greatly increased by eating certain vegetables, such as rhubarb and tomatoes, and also by the defective oxidation of carbohydrates. Pathologically it is often much increased in diabetes mellitus.

Test for oxalic acid. Take 1000 c.cm. of urine, add a sufficiency of 10 per cent. solution of calcium chloride and of liquor ammoniae, and then make acid with acetic acid. A precipitate will fall, consisting of calcium oxalate and uric acid mixed. To encourage complete separation out of the oxalate, the mixture should stand for twenty-

four hours. Filter, and wash the precipitate retained on the filter paper with dilute hydrochloric acid. This dissolves the oxalate, and carries it through in solution, the uric acid being left behind. The filtrate is now treated with ammonia, when the calcium oxalate is once more precipitated. Crystals of this salt occurring in urine naturally, are readily recognized by the microscope.

Dextrose ($C_6H_{12}O_6$) may be regarded as an almost constant constituent of normal urine, although it is present in very small quantities, *e.g.* 1 or 2 grains in twenty-four hours. Its detection is accomplished by isolating it by means of benzoyl chloride, but this is a laboratory method and has no clinical value.

Animal-gum, a dextrin-like body, also occurs in traces in the urine in health; it does not reduce Fehling's solution till after it has been boiled with a mineral acid.

Inosit ($C_6H_{12}O_6$) or muscle sugar may also be found in the urine in health, especially after copious ingestion of liquid. It does not ferment, nor does it reduce copper salts.

Lastly, traces of the *volatile fatty acids*, such as acetic and butyric, may be present, and frequently are, in perfect health.

Our study of the normal organic constituents of urine has thus shown us that only some six or seven are of special importance in practical medicine, the others being of interest mainly from the physiological point of view.

Those of importance are urea, uric acid, the pigments,

the indigogens, the ethereal sulphates, and, in a lesser degree, creatinin, and the student should make himself acquainted with the qualitative tests for all these (except perhaps the pigments), and with the quantitative estimation of urea and uric acid.

CHAPTER III.

The inorganic constituents of normal urine—Chlorine—Sulphuric acid—Phosphoric acid—Carbonic acid—Sodium—Potassium—Ammonia—Calcium—Magnesium—Iron.

Chlorine. This element exists in urine to the extent of from 6 to 9 grms. (average 7·5) in the daily excretion of a healthy adult. It is altogether in the form of chlorides, chiefly the sodium salt. The main source of this element is the food, most of the chlorides being ingested preformed. The amount is therefore increased if a large amount of common salt be taken by the mouth.

In disease it is particularly affected in pneumonia and to a less extent in pleuritic and other effusions; also in marked diarrhoea and sweating, and in chronic disease of the kidneys. In pneumonia the chloride excretion may sink very low, from the amount, it is supposed, exuded during hepatization, and it begins to rise again as the state of the patient improves and absorption takes place. R. Hutchison has studied this matter with care, and looks upon the chloride excretion in pneumonia as of signal use in prognosis and diagnosis.

Tests for chlorides. (1) *Qualitative.* To 5 c.cm. of urine in a test-tube add a few drops of dilute nitric acid, and

then a few drops of 1 per cent. solution of silver nitrate. A curdy white precipitate of silver chloride forms at once, insoluble in dilute mineral acids, but readily dissolved by solution of ammonia. If the urine contain albumin it should first be removed, as silver will precipitate it and might thus cause an error.

(2) *Quantitative estimation.* A rough approximation of the amount of chlorides may be made from the amount of precipitate thrown down, care being taken to add excess of the silver nitrate.

For more exact determination we may employ either Mohr's or Volhard's method.

(a) *Mohr's method.* To perform this we require a standard solution of silver nitrate, of such strength that 1 c.cm. will exactly precipitate a given amount of sodium chloride. To make it, dissolve 29.075 grms. of pure fused silver nitrate in 1000 c.cm. of distilled water; of this, each c.cm. will be equivalent to 0.01 grms. of sodic chloride.

To perform the estimation take 10 c.cm. of urine free from albumin, dilute it with 90 c.cm. of water and place in a porcelain dish. It is necessary that we have an indicator to let us know when all the silver chloride is precipitated, so we add to the diluted urine a few drops of a saturated solution of potassium chromate (K_2CrO_4), *not bichromate*. This gives a reddish precipitate with silver nitrate.

Having filled a burette with the standard silver solution, we run the latter into the diluted urine, which at once becomes opaque from the precipitated silver chloride. We continue adding the standard solution, stirring the while,

till a reddish tint appears. This indicates that all the chloride is precipitated, as the red silver chromate is not formed till this occurs. We now read off from the burette how much of the standard solution has been used, and deduct 1 c.cm., as a little of it always goes to form other silver compounds before the chromate is precipitated.

Ex. Suppose we used 8 c.cm. of the standard solution; deducting 1 c.cm. that leaves 7, each equivalent to 0.01 grms. sodic chloride.

$0.01 \times 7 = .07$ grms. in 10 c.cm. urine, or 0.7 grms. in 100. If the patient were passing 1200 c.cm. per diem, the total chloride as sodium salt would be 8.4 grms.

If it be desired to state it as chlorine, then :

$\text{NaCl}(58.5) : \text{Cl}(35.5) :: 8.4 : 5.09$ grms. chlorine.

Of sodium chloride 11–12 grms. are excreted daily in health.

(*b*) *Volhard's method.* In this the chlorides are all precipitated by a measured excess of the standard solution of silver nitrate, and then the quantity of silver still remaining as nitrate is estimated by means of a standard solution of ammonium sulphocyanide, which forms an insoluble silver sulphocyanide. We ascertain in this way how much silver nitrate is still left uncombined, and, knowing the quantity we took to begin with, we arrive at once by a process of simple subtraction at the quantity that must have gone into combination as chloride, and so the chlorine can be easily calculated.

In carrying out the process we use a solution of silver nitrate of the same strength as that employed in Mohr's method, viz. 1 c.cm. = 0.01 grms. sodium chloride. We take 15 c.cm. of this solution, 10 c.cm. of urine, 4 c.cm.

of pure nitric acid, and 50 c.cm. of distilled water, and shake together in a small flask; water is then added till the whole measures exactly 100 c.cm., when it is shaken again. A precipitate of silver chloride forms at once, and after it has settled down, it is filtered off. Of the filtrate we take 50 c.cm. (corresponding to half the quantity of urine employed), and add a few cubic centimetres of a strong solution of iron-ammonia alum to act as an indicator, and then run in from a burette a standard solution of sulphocyanide of ammonium (6.495 grms. to the litre), of which 2 c.cm. are equivalent to 1 c.cm. of the silver solution. As soon as all the silver has gone into combination with the sulphocyanide, the iron-alum will strike a red colour with the latter, and we know that the end reaction has been reached.

Ex. Suppose for 50 c.cm. of the filtrate, 6 c.cm. of the sulphocyanide solution be required; since 2 c.cm. of it are equal to 1 of the silver solution, there are obviously 3 c.cm. of silver solution left in the 50 c.cm.; as this corresponds to half the urine, it gives a result of 6 c.cm. for the total urine taken. But 15 c.cm. of the standard solution were used at first, therefore,

$$15 - 6 = 9 \text{ c.cm. gone into combination as chloride.}$$

So $0.01 \times 9 = 0.09$ grms. sodic chloride in 10 c.cm. urine.
 $= 0.9$ grms. sodic chloride per cent. Or if the patient pass 1200 c.cm. of urine in the day, a total excretion of 10.8 grms.

(c) A third method of estimating chlorides is Liebig's, which need not be described in detail.

Sulphuric acid (H_2O , SO_3). This occurs in urine chiefly in combination with sodium and potassium, but

as mentioned on p. 45, one tenth of the sulphuric acid excreted by the kidneys is in the form of conjugated sulphates. The chief source of this acid is not the food, which contains only a small amount of sulphates, but the body itself, most of it being derived from the oxidation of proteid compounds containing sulphur. The total amount excreted per diem is 1·5 to 3 grms. It is increased occasionally in fevers. Of the total sulphur in urine, only 80 per cent. is in the form of sulphuric acid, 20 per cent. existing as neutral sulphur compounds, *e.g.* traces of cystin, potassium sulphocyanide, and sulphur compounds derived from bile.

Tests for sulphates. (1) *Qualitative.* To 5 c.cm. of urine in a test-tube add a few drops of dilute hydrochloric acid to keep the phosphates in solution, and a few drops of a 10 per cent. solution of barium chloride, when an abundant white precipitate of barium sulphate will at once appear. To demonstrate the presence of both inorganic and aromatic sulphates the student is referred to p. 46, where this point is discussed.

(2) *Quantitative test.* To determine the total sulphuric acid as SO_3 , we make use of a standard solution of barium chloride, prepared by dissolving 30·5 grms. of the pure crystals in one litre of water. Of this solution 1 c.cm. will exactly precipitate 0·01 grms. of anhydrous sulphuric acid (SO_3).

Place 100 c.cm of urine in a beaker along with 5 c.cm. of pure hydrochloric acid, and boil the whole to break up the conjugated sulphates. The barium chloride solution must now be run in from a burette as long as any precipitate falls down. We cannot make use of any indi-

cator in the fluid to show when the sulphates are all precipitated, but we may, from time to time, allow the precipitate to settle, and then remove a few drops of the clear fluid lying over it by a pipette, and place them in a watch-glass over a dark surface. To this, a drop or two of the solution from the burette may be added, and if any further precipitate appear, we know that all the sulphuric acid is not yet thrown down, and having returned the contents of the watch-glass to the beaker, we run in a little more of the standard solution from the burette. If we fear too much has been run in, we can test this by taking a little of the clear liquid from the beaker, placing it in a watch-glass, and adding a drop or two of 20 per cent. solution of potassium sulphate. If there be excess of barium chloride, a precipitate of the sulphate will occur. Having reached the exact point, we read off the amount of standard solution used, and as each c.cm. = 0.01 gm. SO_3 , we can quickly calculate the total amount in 100 c.cm. of urine.

Ex. Suppose 16 c.cm. of the standard solution are used, then $16 \times 0.01 = 0.16$ grms. SO_3 in 100 c.cm. If the patient passed 1400 c.cm. of urine the total would be $0.16 \times 14 = 2.44$ grms. per diem. Variations in the amount are of no great practical importance.

Phosphoric acid. This is always present in urine as common or orthophosphoric acid ($3\text{H}_2\text{O}$, P_2O_5 or H_3PO_4), and exists in two varieties of combination: (1) as phosphates of the alkaline metals, mainly of sodium, and (2) as phosphates of the alkaline earths, magnesium and calcium, often called earthy phosphates. The sodium salt in healthy fresh urine is sodium di-hydrogen phosphate

(NaH_2PO_4), and to this the urine owes most of its acidity; the potassium salt, which is scanty, is potassium dihydrogen phosphate (KH_2PO_4), while the earthy phosphates are acid calcium and acid magnesium phosphates ($\text{Ca}(\text{H}_2\text{PO}_4)_2$, and $\text{Mg}(\text{H}_2\text{PO}_4)_2$). If the urine be neutral there may be present di-sodium hydrogen phosphate (Na_2HPO_4) and di-calcium and di-magnesium phosphates (CaHPO_4 and MgHPO_4). If the fluid be alkaline we may find tri-sodium, tri-magnesium, and tri-calcium phosphates (Na_3PO_4 , $\text{Ca}_3(\text{PO}_4)_2$, and $\text{Mg}_3(\text{PO}_4)_2$), and in cases of putrefactive change, ammonio-magnesium phosphate ($\text{NH}_4\text{MgPO}_4 + 6\text{H}_2\text{O}$), erroneously termed triple phosphate.

All the varieties of the alkaline phosphates are soluble in water, but only the acid salts of calcium and magnesium.

It will be readily gathered from the above that the phosphates in urine can easily change from one form to another. An example of this is frequently seen when testing for albumin. If the urine be but slightly acid, a cloudiness may appear on boiling, simulating albumin, from which it is readily distinguished by its solubility in acetic acid. The explanation that has been given of this is, that there is present at first the di-calcium phosphate mentioned above, which under the influence of heat is decomposed into soluble acid calcium phosphate and insoluble tri-calcium phosphate, which is precipitated, thus:

$$4\text{CaHPO}_4 = \text{Ca}(\text{H}_2\text{PO}_4)_2 + \text{Ca}_3(\text{PO}_4)_2.$$

The magnesium salts behave similarly.

The phosphoric acid in urine is mainly derived from the phosphates in the food; part of it, however, arises in the body from oxidation of phosphorus, containing tissues such as nuclein and lecithin. It is more abundant during

a flesh diet than during a vegetable one. The total amount excreted daily, estimated as P_2O_5 , varies from 2.5 to 3.5 grms., three-fourths of which is united to the alkalies and one-fourth to the alkaline earths.

It is increased in fevers, in diabetes, in some cases of osteo-malacia and other diseases of bones, and in destruction of nervous tissue. It is said to be diminished during pregnancy and in kidney disease.

Tests for phosphoric acid. (1) *Qualitative.* To demonstrate the earthy phosphates, take 5 c.cm. of urine in a test-tube and make strongly alkaline with liquor ammoniae. A white precipitate of the phosphates of calcium and magnesium is produced, and if the tube be allowed to stand for twelve hours the whole of these salts is thrown down, the alkaline phosphates retaining their solubility.

If the precipitate be filtered off, and sufficient nitric acid added to the filtrate to make it distinctly acid, the addition of a few drops of a 10 per cent. solution of ammonium molybdate will cause a yellow precipitate of ammonium phospho-molybdate. Both kinds of phosphates respond to this test.

(2) *Quantitative analysis.* This is usually performed clinically by means of a standard solution of uranium nitrate, which will precipitate the phosphoric acid. The solution is made by dissolving 35.5 grms. of crystalline uranic nitrate in 1000 c.cm. of distilled water; each c.cm. of this solution is equivalent to 5 mgrs. of anhydrous phosphoric acid (P_2O_5). We also require an acid solution of acetate of sodium, made by dissolving 10 grms. of pure sodium acetate in 60 c.cm. of distilled water, adding 10 c.cm. of glacial acetic acid, and making up the

whole to 100 c.cm. with distilled water. As an indicator a 10 per cent. solution of potassium ferrocyanide is used. To perform the estimation, 50 c.cm. of the urine are placed in a porcelain dish, along with 5 c.cm. of the sodium acetate solution, and heated on wire gauze by means of a spirit lamp to just short of boiling-point, say 85° C. A burette having been filled with the standard solution, the latter is allowed to run into the hot urinous mixture. The indicator is not added to the mixture, but is placed in a series of drops on a porcelain slab or other white surface, and from time to time a drop of the urine is placed on one of the drops by means of a glass rod. As long as there is no change in colour, we know that enough uranium solution has not been added, as the metal has more affinity for the phosphates than for the ferrocyanide, and will not unite with the latter till the former is all used up. If the urine in the dish cool at all during the process, it must be heated up again, as the combination of uranium and phosphoric acid only takes place perfectly near boiling-point, and the mixture must also be stirred frequently. The sodium acetate is there for the purpose of presenting a base for the nitric acid set free from the uranic nitrate to act upon, as free nitric acid must not be present though free acetic acid may. At length a chocolate-brown colour is struck by one of the drops of indicator, and we know the end-reaction has been reached, and may now read off the number of c.cm. of standard solution used.

Ex. Suppose 23.7 c.cm. have been used; as each is equivalent to 5 mgrs. or 0.005 grms. P_2O_5 , this indicates that 0.118 grms. P_2O_5 are present in 50 c.cm. of urine.

If the whole day's urine measure 1370 c.cm. the total excretion of phosphoric acid will be

$$\frac{0.118 \times 1370}{50} = 3.23 \text{ grms.}$$

A trace of phosphorus probably occurs in the urine in organic combination in the form of glycerophosphoric acid.

Carbonic acid (CO_2). This may occur free in a loose form in acid urine, easily dissipated by boiling. When the urine is alkaline, the gas is fixed in the shape of alkaline carbonates, occurring abundantly in the urine of herbivora as the result of the metabolism of the organic acids and their salts, in their food.

Sodium and potassium. The sodium in urine is chiefly in the form of chloride, but part of it exists in combination with phosphoric, uric, and sulphuric acids; from 10 to 11 grms. are excreted daily. Potassium occurs to the extent of from 2 to 3 grms. per diem, chiefly as the chloride; it is, at least relatively, increased during fever.

Ammonia. A little free ammonia occurs in fresh urine, and a little more in combination, *e.g.* with uric acid; about 0.7 grms. (12 grs.) is excreted in twenty-four hours. It may be demonstrated by taking 25 c.cm. of urine in a small beaker, adding 10 c.cm. of milk of lime, and covering the vessel with a sheet of glass to the under surface of which a strip of moistened red litmus-paper has been made to adhere. The lime liberates the volatile ammonia which very speedily turns the red litmus-paper blue.

Schlösing's method is to add the milk of lime to 25 c.cm. of urine, and suspend within the beaker a small capsule containing dilute hydrochloric acid, the whole being covered by a bell-glass. The acid absorbs the discharged ammonia, forming the chloride. After twenty-four hours standing, the latter may be evaporated, extracted with water, and tested with tetra-chloride of platinum, which gives a yellow precipitate.

Calcium and Magnesium. These metals occur in but small quantities (about 0·2 to 0·3 grms. per diem), and exist chiefly in combination with phosphoric acid.

Iron. Quite minute traces of iron occur in normal urine to the extent of from 0·001 to 0·01 grms. in the whole day's excretion.

CHAPTER IV.

Pathological elements in urine—Proteid bodies—Serum-albumin—Serum-globulin—Albumoses—Peptones—Nucleo-albumin—Mucin—Fibrin.

ALTHOUGH as mentioned already (p. 47), minute traces of a proteid body occur in healthy urine, this may be disregarded for all practical purposes, and we are entitled to say when the urine responds to the tests for, let us say, albumin, that there is present a deviation from the normal, whether it be transitory as in many cases of functional disturbance, or whether it be more or less permanent, as in organic kidney and heart disease.

Patients exhibiting this condition are said to have 'albuminuria,' and the urine in such cases is found to contain two native proteids, called serum-albumin and serum-globulin. A brief account of these and of their differential tests will first be given, but the student should bear in mind that in testing for albumin in urine, we do not distinguish between these two bodies as a rule, but apply tests to which both of them react.

General reactions of proteids. To test these the student may use a solution of white of egg (1 in 10), which contains both albumin and globulin.

(1) They coagulate on boiling, after slight acidulation with acetic acid.

(2) The addition of nitric acid causes a white precipitate, which becomes yellow when boiled (xantho-proteic reaction).

(3) The addition of a single drop of 1 per cent. solution of copper sulphate and some liquor potassae produces a violet coloration.

(4) The addition of acid nitrate of mercury (Millon's reagent) gives a white precipitate, becoming red on boiling.

(5) The addition of an equal volume of 10 per cent. solution of trichloroacetic acid produces a very complete precipitate.

There are many other reactions for the native proteids, but these suffice to distinguish them.

Tests for serum-globulin alone. This body reacts to all the above-mentioned tests, and in addition :

(1) If a little be dropped into a vessel of distilled water, a cloudiness becomes apparent, as serum-globulin requires the presence of salts to keep it in solution.

(2) If the fluid be saturated with crystals of magnesium sulphate in the cold, all the globulin is precipitated; albumin if present is not.

(3) If to the fluid there be added an equal volume of a saturated solution of neutral ammonium sulphate (which makes the whole liquid half-saturated), the globulin is precipitated; albumin is not. The two can thus be separated by these means, plus filtration.

Tests for serum-albumin. (1) Saturation in the cold with crystals of neutral ammonium sulphate will precipitate the albumin; it will, of course, also precipitate serum-globulin if present.

In albuminuria, as already stated, we find both these bodies present and in varying proportions. In some quantitative estimations which the author made, he found such proportions of globulin to albumin as 1 to 3, 1 to 7, 1 to 12.

They may arise from the admixture of blood, pus, chyle, semen, etc., with urine, but in such cases the amount present is usually small, and considerable amounts as a rule are present only in cases where the albumin occurs in the urine from the moment it leaves the kidney.

In albuminuria the amount present may vary greatly, from 0.05 to 1 per cent., or from 0.5 to 15 grms. per diem. Quantities under 2 grms. a day may be looked upon as small, and above 8 grms. as large, according to Tappeiner.

As regards the conditions of disease in which albumin occurs in the urine, the following may be mentioned as the chief: acute nephritis, chronic renal inflammation, amyloid disease of the kidneys, organic heart-disease, many infective fevers, notably scarlatina, erysipelas, diphtheria, pneumonia, and puerperal fever; in pyrexia generally, in lead-poisoning, syphilis, pregnancy, and the puerperal state. It may occur, however, and often does, in many other forms of disease.

Functional albuminuria is that variety which occurs under special conditions of diet, exercise, etc., and as a rule, without any co-existing impairment of general health. It is frequently noticed in the morning or forenoon. As Osler says, the most striking feature it presents is its variability.

Tests for albumin in urine. Albuminous urine is often a little opalescent and often froths more easily than the

normal excretion. Before applying any tests, it may be advisable that suspended bacteria, etc., be removed. This cannot be done by ordinary filtering as bacteria pass through the pores of filter-paper, but may be accomplished by shaking up some urine in a test-tube with a small quantity of kieselguhr (infusorial earth) or calcined magnesia. On filtering now, a clear filtrate will be obtained, to which tests may be suitably applied.

In all cases it is advisable to have a sample of the urine in a second tube for the sake of comparison, to which no reagent has been added, or if the reagent require to be boiled, one in which the reagent has been added but not boiled. This will be found of distinct service where the quantity of albumin is small.

Tests. (1) *The cold nitric acid or Heller's test.* Into a clean test tube 2 or 3 c.cm. of pure strong nitric acid are introduced; the tube is then held in an oblique position, and by means of a pipette, 5 c.cm. of urine are allowed to trickle gently down the side of the tube. The lighter urine floats on the top of the acid, and at the interface formed by the two liquids there appears either at once, or in a few minutes, a distinct ring-like opacity if albumin be present.

This test, like certain others, is based on the fact that many chemical bodies can coagulate albumin. Some authorities prefer to put the urine in the tube first, and let the nitric acid trickle down past it, raising it upon its surface. In the author's opinion no advantage is to be derived from so doing.

This is a very excellent test, but, as is the case with certain of the others, there are some fallacies that may

arise here; in the author's opinion the importance of such fallacies is often much exaggerated, but it is at least right that the observer should be made cognizant of them.

Fallacies. (a) If the urine contain much *urea*, either because the former is concentrated or because the urea itself is very abundant in quantity, a precipitate of crystals of nitrate of urea may form on adding the urine to the acid or *vice versâ*. The precipitate often assumes the form of a round cake, the crystals being deposited in layers. The appearance of such a phenomenon is determined by various factors, such as the amount of urea present, the temperature (occurring more readily in the cold), the strength of the acid (more quickly the stronger it is), and, lastly, by time, sometimes appearing at once, sometimes requiring a few minutes for its development. Any doubt can be readily solved by diluting a second sample with, say, half its volume of tepid water, and repeating the test; the crystals also dissolve with gentle heat.

(b) *Uric acid* if present in considerable amount may be thrown out of solution by nitric acid and form a ring-like haze. This, however, generally appears at some little distance above the contact surface of the fluids, and doubt may here also be solved by diluting the urine first.

(c) If the patient has been taking *drugs* like *copaiba*, *cubebs*, etc., the resinous acids which they contain, and which find their way out of the system in the urine, may be precipitated by nitric acid. As these acids are soluble in rectified spirit, the addition of a little of this reagent will cause their disappearance.

(d) *Albumoses* (not true peptones) may be present and form a haze in the urine with nitric acid. This precipitate is soluble on heating and reappears again when the liquid is cool.

(e) *Mucin* gives a diffuse haze with cold nitric acid, and nucleo-albumin a sharper ring; differential tests for these two bodies will be found at p. 74.

(2) *Boiling with the addition of acetic acid.* This is a good and delicate test if carried out carefully. Sometimes nitric acid is used instead of acetic, and to this there is no objection. It is occasionally advised that the acid be added first and the boiling accomplished thereafter; but Finlayson¹ has pointed out various objections to this procedure, and places chief reliance on boiling with subsequent delicate acidulation. To carry out the test, 5 c.cm. of the urine should be brought just to the boiling point in a test-tube, and then a drop or two (not more) of acetic acid added. If albumin be present the liquid may become turbid on boiling, and will certainly become so when the acetic acid is added. There is no need to add more than one or two drops of acid unless a turbidity of phosphates has appeared on boiling, when enough must be added to dissolve this. The explanation of this precipitation of phosphates has been given already (p. 56). If the urine be turbid from urates to begin with, the application of heat will remove this.

By applying both the cold nitric acid test and that just described, we can assure ourselves with certainty whether or not albumin be present, and in actual practice need not pursue further methods; it will, however, be

¹ *Clinical Manual*, 3rd edition, 1891, p. 495.

advisable to mention several other tests which are often regarded with favour.

(3) *Ferrocyanide of potassium and acetic acid test.* This is another sensitive test, and beset by few fallacies. To carry it out add to 5 c.cm. of urine in a test-tube, a few drops (4 or 5) of a 10 per cent. solution of potassium ferrocyanide in the cold, and then an excess of acetic acid, when a turbidity quickly appears in the presence of albumin. If we please we may mix the ferrocyanide and acetic acid together first, and place this in the tube, and allow the urine to flow down and rest upon it. An opaque ring is formed at the interface between the fluids. Any albumoses present are also precipitated by this test but are redissolved again on heating.

(4) *Picric acid test.* Picric acid or tri-nitro-phenol, is another useful reagent for the detection of albumin. In employing it we make use of a saturated watery solution which, on account of its density being lower than that of urine, may be floated on to the surface of 5 c.cm. of urine in a test-tube. The urine may first be acidulated with citric acid, but this is not absolutely essential. If albumin be present, a whitish-yellow coagulation ring forms where the fluids touch.

Another way of performing the test is to drop small crystals of picric acid into the urine, when a little cloud of coagulation will be formed by each particle as it sinks through the urine.

The albumoses also give a precipitate with picric acid, which dissolves on heating, and reappears on cooling.

Alkaloids, particularly quinine, give a precipitate with

this acid, so inquiries should be made as to any drugs the patient may be taking.

In addition to the above-mentioned tests, the following reagents precipitate urinary albumin: Metaphosphoric acid, trichloracetic acid, sodium chloride + acetic acid, potassium-mercuric iodide + citric acid (Tauret's reagent), and other bodies. Of these trichloracetic acid ($\text{CCl}_3\text{CO}_2\text{H}$) is, according to Allen,¹ probably the most sensitive; a saturated solution in the cold is said to be able to show 1 in 100,000. It also throws down quinine and other alkaloids as a dense white precipitate, soluble on heating.

Spiegler's reagent (perchloride of mercury 8 grms., tartaric acid 4 grms., sugar 20 grms., and water 200 c.cm.) is another delicate test for albumin in urine; the latter should be acidulated, filtered, and floated on to the reagent in a test-tube.

For portability Oliver has suggested the use of slips of paper in two sets—one soaked in solution of citric acid, the other in solution of ferrocyanide of potassium and dried. One of each of these is treated with a little water and the urine added drop by drop.²

Quantitative estimation of albumin. (1) A rough approximation may be made after boiling with acidulation, from the quantity of precipitate that collects at the bottom of the tube after the latter has stood some hours.

(2) *Precipitation and weighing.* To 50 c.cm. of urine is added an equal volume of 10 per cent. trichloracetic

¹ Allen, *Chemistry of Urine*, 1895, p. 113.

² The papers may be obtained from Wilson & Son, Harrogate, and the student is referred for further details to Dr. Oliver's book *Bedside Urine Testing*, 4th ed.

acid, which will thoroughly precipitate all the serum-albumin and serum-globulin. The precipitate is collected on a filter-paper of known weight, washed with boiling water, dried, and again weighed, when the quantity of coagulable proteids in 50 c.cm. of urine will be ascertained, and, if we multiply by two, the percentage. This method requires the use of a chemical balance, and therefore is hardly suited for clinical work.

(3) *Esbach's method.* This is a favourite clinical method, and is often made use of. In it we employ a special tube or albuminometer, some 16 cm. high and 2 cm. in diameter, which has a line on it, in its upper part, marked *R*, and below it one marked *U*. In the lower half there is a graduated scale representing grammes of dried albumin per thousand (fig. 6).

To use the tube fill it with urine up to the mark *U*, avoiding frothing, and then with Esbach's reagent (see p. 15) up to the line *R*. The tube must be well corked and shaken two or three times in a horizontal position to ensure thorough mixing, but, at the same time, to avoid frothing. The corked tube may now be set aside for twenty-four hours, and at the end of that time we read off on the scale the height to which the precipitate reaches.

Ex. Suppose the deposit of albumin reaches up to the line 2.5; this indicates that 2.5 grms. are present in one litre of urine, or 0.25 per cent. If the patient were passing

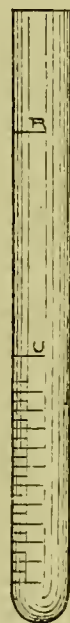


FIG. 6. —ESBACH'S ALBUMINOMETER.

1320 c.cm. per diem the total would be $0.25 \times 13.2 = 3.3$ grms. of albumin. If there be any reason to suspect that the urine contains a great deal of albumin, or if an estimation show it to be above four parts per thousand, the process should be repeated with the urine diluted with an equal volume of water (or two volumes if necessary), and the final result multiplied by two or three as the case may be. In any case, if the specific gravity be over 1010, it is safe to dilute the urine till the density is reduced to this figure, or even a little below it. The results obtained by Esbach's process are tolerably accurate, but not with small amounts of albumin.

(4) *Robert's and Stolnikow's method modified by Brandberg.* This method is based on the fact that in the cold nitric acid test, the ring of albumin appears the quicker the more of that substance there is present. It has been found experimentally that if 0.0033 per cent. of albumin be present the ring appears in two to three minutes. By making a series of dilutions of definite strength of an unknown urine, an estimate may be made of the quantity of albumin present in it. For example, if the urine show the ring of coagulation in two to three minutes when diluted 10 times, it indicates that it contains $0.0033 \times 10 = 0.033$ per cent. of albumin; if when diluted thirty-fold it shows the same, it must contain $0.0033 \times 30 = 0.1$ per cent., and so on, for the more albumin there is present the greater must be the degree of dilution to produce the ring at the proper time.

In estimating the amount present in any unknown specimen, it is necessary first of all to make certain dilutions of it, and Tappeiner recommends that to begin with, dilutions of 10-fold, 30-fold, and say 150-fold, should be made.

If now, after trying these specimens with the acid, it is found that the ring appears in less than two or three minutes in the former two, and in more than three minutes in the 150-fold dilution, the proper point will be reached at a dilution somewhere between 30-fold and 150-fold. We may find it, let us say, at a dilution of eighty-fold; the percentage of albumin will then be $0.0033 \times 80 = 0.26$. In any case the amount of albumin per cent. is obtained by multiplying 0.0033 by the degree of dilution.

Albumoses and Peptones. The term peptones, as formerly employed by Brücke, included not only true peptones but also the albumoses, *i.e.* the intermediate bodies between native proteids and peptones, developed during digestion. By Kühne's peptones, on the other hand, are meant only true peptones which are not precipitated by various reagents that readily precipitate albumoses. When albumoses occur the term "albumosuria" is often used now to distinguish it from "peptonuria," for it is the opinion of most clinicians at the present time that true peptones occur very rarely in urine, and Stadelmann even says that they never occur at all in the fresh excretion, but may appear in old albuminous urine as the result of the action of micro-organisms on the albumin. Albumoses may find their way into the urine either from being absorbed from the alimentary canal in cases where ulceration exists, or from being developed elsewhere in the body, *e.g.* in deep-seated abscesses.

The chief diseases in which albumosuria occurs are deep-seated suppurations, croupous pneumonia, osteomalacia, multiple myelogenous tumours, and sarcomata. If persistently present it is to be regarded as a bad

symptom. Rosin reports a case lately where albumoses were plentifully present, and where the diagnosis of Bright's disease was made; the patient, a woman aged 36, died, and the autopsy revealed the presence of multiple sarcomata of the ribs. Transient albumosuria may occur, *e.g.* in involution of the puerperal uterus, and is not regarded as of serious import.

Reactions of albumoses and peptones. Albumoses, of which proto- and deuterio-albumose are the chief examples, are soluble in water hot or cold, but are precipitated by alcohol, meta-phosphoric acid and tannic acid. Both proto- and deuterio-albumose give a precipitate with ferrocyanide of potassium and acetic acid, and with picric acid, the precipitate in both cases disappearing on heating and reappearing on cooling. Nitric acid in the cold gives a precipitate with proto-albumose, soluble on heating, but not with deuterio-albumose till excess of sodium chloride has been added.

Neutral ammonium sulphate added to saturation in the cold, precipitates all the proto- and most of the deuterio-albumose. Both of them exhibit the biuret reaction (rose-pink colour) on adding a drop of dilute copper sulphate solution and excess of caustic potash. True peptones also give the biuret reaction and are precipitated by alcohol, phosphotungstic acid, and tannic acid; they are also precipitated by saturation with neutral ammonium sulphate at boiling point (Devoto's method).

Application of the tests to urine. Any albumin that may be present must first be entirely removed by boiling with the addition of a little acetic or nitric acid and filtering while hot. Or the urine may be treated with an equal

volume of 10 per cent. trichloroacetic acid solution, heated, and filtered hot. In either case the filtrate will contain the albumoses (and peptones), and may be tested, (1) by the biuret reaction, (2) by the addition of picric acid which gives a precipitate that disappears on heating, or (3) by adding saturated solution of common salt and then nitric acid. If it be desired to test for peptones alone, the urine may be placed in a dialyser for an hour or two, in a vessel of distilled water; practically no proteid body but true peptone will pass out in this time. The water, which will contain the peptone in solution, may be concentrated by evaporation, and subjected to the biuret test.

But it has been pointed out quite recently by Salkowski and others that the biuret reaction for albumoses and peptones in urine is not quite reliable, as urobilin also responds to this test. Bang¹ has recently proposed the following test, which is not difficult of performance.

Eight grms. of neutral ammonium sulphate are added to 10 c.cm. of urine in a small beaker, dissolved with the aid of heat if required, and the whole brought quickly to the boil. The albumoses and peptones are thus precipitated. The mixture is then placed in a centrifuge for a minute, the supernatant liquid separated from the sediment, and the latter rubbed up with absolute alcohol, which has the effect of removing any urobilin present, in solution. The alcohol having been removed, the remainder is dissolved in water, boiled and filtered. If the filtrate respond to the biuret test, albumoses or peptones are present.

¹ Bang, *Deutsch. med. Wochens.*, No. 2, s. 17, 1898.

Harris¹ has devised a delicate test in which, after precipitation of any other proteids present by means of salicyl-sulphonic acid and plumbic chloride, the filtrate (which contains the albumoses and peptones) is treated with a few drops of saturated solution of salicyl-sulpho-tungstate of sodium. A cloudiness indicates the presence of the abnormal bodies, and the test is said to be so delicate that it can reveal them if present to the extent of only 1 in 50,000.

Nucleo-albumin and mucin.—These two proteid bodies may be conveniently studied together. Many authorities now believe that the former is the chief element in the so-called “mucus-cloud” seen in healthy urine; others say both bodies are components of it. Mucin is certainly present in increased amount in cases of cystitis and catarrhal affections of the genito-urinary tract. Both of the substances are insoluble in water. The following are their principal reactions:

(1) *Nucleo-albumin.* (a) A precipitate is given with acetic acid.

(b) With Heller’s test a sharp ring is developed.

(c) A saturated solution of magnesium sulphate gives no precipitate.

(d) No reducing substance is yielded on boiling with a dilute mineral acid.

(e) The ash of nucleo-albumin contains phosphorus.

(2) *Mucin.* (a) A precipitate is given with acetic acid; if testing urine for it, dilute it first with water.

(b) It is soluble in weak alkalies, *e.g.* lime-water.

(c) Heller’s test gives a diffuse haze.

¹ Harris, *Amer. Jour. Med. Science*, vol. exi., p. 557, 1896.

(d) A saturated solution of magnesium sulphate precipitates it.

(e) It yields a reducing body on boiling with a dilute mineral acid. For this reason it is sometimes called a glyco-proteid.

(f) Its ash is phosphorus-free.

If any doubt exist as to whether pus or simply mucin be present, the microscope should be used.

Fibrin. It occasionally happens that the urine contains fibrin, and when it is passed and has cooled, the latter may set like a jelly, or, short of this, may form a kind of sediment. This condition is termed "fibrinuria," and it occurs in cases of haemorrhage into the urinary tract, and sometimes in patients who have had fly-blisters applied or have taken cantharides internally. Owing to the presence of blood, the urine may have a reddish tint.

Test for fibrin. It is insoluble in water, in dilute acids, and in neutral saline solutions; it dissolves gradually in strong mineral acids, forming acid-albumin, and, if placed in 2 per cent. solution of hydrochloric acid, swells up. The subsequent addition of pepsin to the latter will quickly peptonize it, if the mixture be kept at 40° C. In the condition named "chyluria," not only fibrin, but also fat and albumin are present in the urine.

CHAPTER V.

Pathological elements in urine continued: Saccharine bodies and their allies — Glucose — Laevulose — Lactose — Pentose — Isomaltose — Glycuronic acid — Acetone — Di-acetic acid — Oxybutyric acid.

Glucose. We have already learned that some sugar (glucose) is present in healthy urine, but the quantity is so small that one may safely say that any urine responding to the ordinary tests for sugar contains that element in excess. Of sugars there are many varieties, but that with which we are specially concerned now, and which constitutes the really pathological element, is glucose or grape-sugar, sometimes called dextrose, since it is dextro-rotatory to a plane of polarized light, in contradistinction to laevulose or fruit-sugar, which is laevo-rotatory, and which may also occur in urine, but which is of quite subsidiary importance. Chemically, glucose is a monosaccharide, with the general formula $C_6H_{12}O_6$, and the constitutional one $CH_2OH \cdot (CHOH)_4COH$, from which we see it is both a primary alcohol and an aldehyde.

When glucose occurs in urine, the condition is termed "glycosuria," and this simply means that grape-sugar is present in the urine, and no more. This condition may

be temporary and unimportant, or it may be permanent and of grave importance. It is met with, according to Osler:¹

(1) Where a large quantity of cane-sugar is ingested, and where part of it, as it were, forces the barrier of the liver and enters the general circulation, or is absorbed directly by the lacteals. This is termed alimentary glycosuria.

(2) In disturbance of the functions of the liver, either produced experimentally as in puncture of the floor of the fourth ventricle, or in disease, as in hepatic cirrhosis (some cases), and especially in diabetes mellitus.

(3) During the administration of certain substances, *e.g.* phloridzin, and

(4) In general metabolic disturbance, as in disease of the pancreas and in gout.

In older text-books glycosuria is sometimes described as occurring after the administration of chloroform and chloral hydrate. It is now believed that it is not glucose that is present in such cases, but a body named glycuronic acid, to be described later on (see p. 90).

The quantity of grape-sugar that may be present in urine varies enormously; there may be only 0·5 per cent., or as much as 5, 6, or even 10 per cent. Or to express it differently, let us say from 1 oz. of sugar up to 1 lb. or more in twenty-four hours.

Tests for glucose. I. *Qualitative.* These are numerous, and vary somewhat in their excellence and their freedom from fallacies. We shall describe the five tests which are of

¹Osler, *Principles and Practice of Medicine*, 3rd. ed., 1898, p. 420.

most importance, and briefly mention some of those of less value.

(1) *Trommer's test.* This depends on the fact that grape-sugar has the power of holding cupric hydrate, $\text{Cu}(\text{OH})_2$, in solution, forming a blue fluid, and further of reducing the cupric hydrate in an alkaline medium on the application of heat, the oxygen taken from the cupric compound going to oxidize the glucose. The cupric hydrate is reduced to a cuprous compound, either the hydrated cuprous oxide, $\text{Cu}_2\text{O} \cdot \text{H}_2\text{O}$, which is yellow, or the anhydrous oxide, Cu_2O , which is red.

We may perform the test in two different ways: (a) Take 10 c.cm. of the suspected urine in a test-tube, add 2 c.cm. of 20 per cent. solution of caustic potash, and then a 5 per cent. solution of cupric sulphate, drop by drop, shaking all the while; as the latter is dropped in, a bluish-white precipitate, $\text{Cu}(\text{OH})_2$, forms, which at once dissolves, producing a blue solution. Go on adding the copper salt till a mere trace of the hydrate remains undissolved and then stop. We have then cupric hydrate in solution in an alkaline medium in the presence of glucose. Or,

(b) To 10 c.cm. of urine in a test-tube a few drops of the cupric sulphate solution are added, and then the caustic potash carefully, with shaking, till the last trace of cupric hydrate is just dissolved. In either case the mixture is now boiled, and it is preferable to apply the heat at the upper part of the column of fluid, when, if sugar be present, the solution grows turbid and dull; then traces of yellow-red appear, contrasting with the blue colour below, and when the whole is boiling, the entire liquid is an opaque red or yellow, according as cuprous oxide or hydrate is formed.

This is a good test, but the reduction of the cupric hydrate may be accomplished by other substances, such as uric acid and urates, creatinin, alcapton, and glycuronic acid, which may be present in urine, and which are termed "reducing bodies" in this connection. The test is fairly delicate, and shows the reaction with 0·5 per cent. of sugar and upwards.

(2) *Fehling's test.* In Fehling's reagent the cupric hydrate is held in solution by means of a tartrate in an alkaline solution, and here again reduction is effected by grape-sugar at the boiling point. The solution is made by dissolving 34·64 grms. of pure cupric sulphate in say 250 c.cm. of distilled water, and then adding enough water to make up the volume exactly to 500 c.cm.; 173 grms. of Rochelle salt are next dissolved either in 480 c.cm. of caustic soda solution of specific gravity 1·12, or in 400 c.cm. of distilled water, to which are next added 51·6 grms. of pure sodium hydrate; the volume here also is made up to 500 c.cm. with water. On mixing together 5 c.cm. of each of these solutions, 10 c.cm. of a fine deep blue fluid is obtained, which is entirely reduced by 0·05 grms. of grape-sugar.

Fehling's solution can of course be made ready for use without mixing, but it is apt to change with time, and to become unreliable, owing, it is said, to the formation of racemic acid ($C_4H_6O_6 \cdot H_2O$), which can itself reduce cupric hydrate. So it is better to prepare it as described above, and to keep it in two separate stoppered bottles as little exposed to light as possible.

Having mixed some of the solution, we first boil it by itself to see whether it is pure and trustworthy; any

alteration in tint or transparency should condemn it. Having satisfied ourselves that the reagent is in good order, we add to it, still hot, 5-10 c.cm. of the suspected urine (first freed from albumin), and boil up quickly again. We may at once see the change of colour due to the reduction of the cupric compound, or it may not appear until the solution has stood for a little. The reagent should always exceed the urine in volume, and it should not be employed with ammoniacal urine; Pavy's test may then be used.

Fehling's test is good and fairly delicate, and can show 0.2 per cent. of sugar; it is affected, however, by the reducing bodies such as uric acid urates, creatinin, and glycuronic acid. This latter objection can be overcome by the procedure advised by Allen.¹ His plan is to treat the urine in neutral or faintly acid solution with cupric acetate, whereby a precipitate of xanthin, hypoxanthin, uric acid, colouring matters, and albumin is produced; a partial precipitation of phosphates and creatinin also occurs. The filtered fluid does quite well for Fehling's test. To put this procedure into effect, take 7 or 8 c.cm. of urine, boil in a test-tube, and add at once 5 c.cm. of the cupric sulphate solution used in preparing Fehling's reagent. A precipitate occurs; when the tube has cooled, add 1-2 c.cm. of a saturated solution of sodium acetate, containing a trace of free acetic acid. A further precipitate now occurs, and, on filtering, a greenish-blue solution is obtained. To this are added 5 c.cm. of the other part of Fehling's test (the alkaline tartrate solution), and the

¹ Allen, *Chemistry of Urine*, 1895, p. 62.

whole boiled for a quarter of a minute. Cuprous oxide at once separates as an orange-yellow precipitate in the presence of one part of sugar in five hundred, or less (0.2 per cent.).

(3) *Böttger's test.* This test is sometimes named Nylander's or Almén's, as the process originally devised by Böttger has been modified by them. It depends on the reduction of a bismuth compound by glucose with the production of the black suboxide. To prepare the reagent in its improved form, dissolve 10 grms. of solid sodium hydrate in 100 c.cm. of water, warm the solution, and then dissolve in it 4 grms. of sodio-potassium tartrate, add 2 grms. of bismuth subnitrate, and let the whole be well shaken. The soda solution acts on the subnitrate to produce a hydrated oxide of bismuth, which is kept in solution by the tartrate. The mixture is finally filtered and kept in a well-stopped bottle in the dark.

To perform the test take 10 c.cm. of the suspected urine first freed from albumin, and 1 c.cm. of the reagent; shake, and boil for two or three minutes. The earthy phosphates are precipitated in any case, but if sugar be present, a fine precipitate of bismuth suboxide forms, and on standing falls to the bottom of the test-tube as a black cloud.

This test is delicate, and is less affected by the reducing bodies in normal urine than Fehling's or Trommer's solutions. It reacts however to rhubarb and senna if these drugs happen to be present, and it also responds to milk-sugar, and to glycuronic acid.

(4) *Phenyl-hydrazine test.* This delicate test may respond to normal urine, as it can detect the presence of 0.1 per

cent. of grape-sugar. In performing it, 5 c.cm. of urine are placed in a test-tube, and to it are added 2 or 3

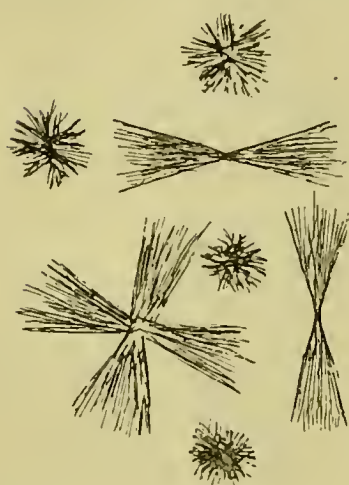


FIG. 7.—CRYSTALS
PHENYL-GLUCOSAZONE.

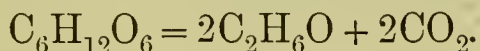
decigrammes (about as much as will lie on the point of a knife-blade) of hydrochloride of phenyl-hydrazine ($C_6H_5HN.NH_2.HCl$), and half as much again of sodium acetate. The tube is put into a boiling water-bath, and kept there for from thirty minutes to one hour. At the end of that time a yellow-brown deposit may already have formed, or, if it has not, will come down on cooling if sugar be present. The deposit

may look quite amorphous, but the microscope shows it to be composed of yellow crystalline needles arranged in bundles or radiating groups; they have a melting point of $205^{\circ} C$ (fig. 7). The deposit may actually be amorphous, and in that case, if we have reason to suspect that sugar is present, we may dissolve the deposit in hot absolute alcohol, dilute with water and boil, to drive off the spirit, when, on cooling, the crystals will appear. The substance formed is named phenyl-glucosazone ($C_{18}H_{22}N_4O_4$). Other sugars also form osazones, but they are not derived from such bodies as uric acid or creatinin, which affect the copper tests for sugar. Laevulose, for example, yields an osazone, so does milk-sugar (phenyl-lactosazone), the crystals in this case melting at a lower point than those of phenyl-glucosazone. This test is not easily performed with milk-sugar in urine. Maltose yields a phenyl-maltosazone, melting at

about 190° C. Its crystals are broader than those of the glucosazone. Galactose also yields an osazone, while cane-sugar gives none.

We may affirm, with hardly any fear of error, that when a urine yields an osazone it contains a sugar, and nearly always this is grape-sugar. The presence of albumin does not interfere with the test. The author has found this method reveal sugar when present to the small extent of 1 in 1000.

(5) *Fermentation test.* In this we take advantage of the power of yeast to convert grape-sugar into alcohol and carbonic acid gas, thus :



This is a good and sure test, and there is no other pathological element in urine that will interfere with it. It is also sufficiently delicate, as it will indicate 0·5 per cent. of sugar, and even less. There are two slight drawbacks to its use, one being that it requires time, and the other that yeast is not always at hand in hospital or private practice.

To perform the test we take a piece of fresh yeast as large as a big pea, and place it in a test-tube, which is filled quite full with the suspected urine and gently shaken. It is always advisable to boil the urine thoroughly for a few minutes beforehand, to drive off any gas that may be dissolved in it. The open end of the tube is then closed with the thumb, and the tube inverted into a small dish of mercury, in which it can be supported by some simple means, such as a test-tube holder fastened to the ring of a retort stand. The whole is then placed in a warm part

of a room, such as a kitchen mantelpiece, and in from 12 to 24 hours a quantity of gas will have collected in the tube if grape-sugar be present. Sometimes a control tube is set up, to show that the yeast is active; in it normal urine, yeast, and a little grape-sugar are placed; sometimes a third tube is used, containing merely normal urine and yeast.

The more sugar present, the more carbonic acid gas is formed. We invert the tube over mercury instead of water, as the gas is soluble in the latter. It has been shown that where very little sugar is present, the gas developed may be dissolved in the urine, and it may be necessary to heat the latter at the end of the process to get the gas off.

The fermentation test cannot be applied to putrefying urine, as the putrefactive processes interfere with the activity of the yeast cells. This test is undoubtedly good and should be resorted to in all cases in which doubt exists.

Besides these five important tests, there are several others that deserve at least a brief notice.

6. *Moore's test.* To 5 c.cm. of urine in a test-tube add one-third of its volume of solution of caustic potash and boil for a few minutes; if grape-sugar be present the fluid assumes a dark yellow or brown tint, due to the oxidation of the grape-sugar.

7. *Picric acid test (Johnson's).* Place 5 c.cm of urine and the same volume of saturated solution of picric acid in a test-tube, add 2-3 c.cm. of caustic potash solution and boil. In the presence of grape-sugar a very dark, almost opaque, red colour is produced, due to the formation of picramic acid.

8. *Indigo-carmin test.* This is the test in the portable urine testing case devised by Oliver of Newcastle. In it there are slips of paper in two sets, one soaked in solution of carbonate of sodium, the other in one of indigo-carmin. One slip from each set is dissolved in 4 c.cm. (about 1 drm.) of distilled water, boiled, and a drop of the urine added, when, if the solution be kept hot, it will change colour, becoming green, red, straw-coloured, and yellow if grape-sugar be present. This depends on the fact that glucose can reduce indigo-blue to indigo-white. If the fluid be now shaken, the oxygen of the air will convert it back again to indigo-blue.

9. In addition to this test that of *Pavy* is also sold in a portable form in the shape of pellets. This test in some ways resembles Fehling's, but ammonia is present too, which has the power of holding the cuprous oxide in solution if any be formed. A pellet dissolved in a little warm water gives a blue solution smelling of ammonia; if a little urine containing glucose be now added and the whole boiled, the fluid becomes, not red as in Fehling's test, but decolorized; if grape-sugar be absent it remains blue. This test has the advantage that it can be used with ammoniacal urine.

II. *Quantitative estimation of sugar.* (1) *By Fehling's test.* We have seen already that 10 c.cm. of Fehling's solution are exactly reduced by 0.05 grms. of glucose, and it is obvious that we can avail ourselves of this fact to estimate the amount of sugar in an unknown solution.

To carry this out, take 5 c.cm. of urine freed first from albumin if any be present, and dilute it with exactly 45 c.cm. of water; this diluted urine (1 in 20) is placed

in a burette. In a porcelain dish are placed precisely 10 c.cm. of Fehling's solution diluted with 40 to 50 c.cm. of water, and this is brought to the boil, on a tripod stand over a spirit lamp. When it is still gently boiling, the diluted urine is run in cautiously from the burette with stirring, when a precipitation of the cuprous oxide begins to take place. After a few c.cm. have been run in, stop for a few seconds to let the precipitate settle, and then note whether the supernatant fluid as seen against the white of the dish still appears blue. If it does, more urine must be added till the blue colour has entirely disappeared. Sometimes it is very difficult towards the end of the operation to say whether all the copper is reduced or not. In that case a very little of the supernatant fluid may be filtered, acidified with acetic acid, and a drop of 5 per cent. solution of potassium ferrocyanide added, when, if any cupric compound still remain, a chocolate brown precipitate of cupric ferrocyanide will come down.

Having at length reached the final point, we read off from the burette the number of c.cm. required, and as they have effected the reduction of 10 c.cm. of Fehling's solution, they must contain 0.05 grms of grape-sugar.

Ex. Suppose 40 c.cm. have been used ; this is equivalent to 4 c.cm. of undiluted urine and contains 0.05 grms. of glucose.

If the patient be passing 5500 c.cm. of urine per diem, the total excretion will be :

$$\frac{5500 \times 0.05}{4} = 68.75 \text{ grms.}$$

If the percentage be desired, then

$$\frac{100 \times 0.05}{4} = 1.25 \text{ per cent.}$$

and this multiplied by 4.375 gives grains per ounce; in this case, 5.46.

Pavy's solution may also be used for estimation, but it is ten times weaker than Fehling's, 10 c.cm. being reduced by 0.005 grms. of grape-sugar.

(2) *The fermentation method.* This also may be used as a quantitative process, since in the development of the carbonic acid gas, the specific gravity of the fermenting urine becomes lowered, and a table of comparisons can be made between the specific gravity and the quantity of sugar originally present.

In performing this method it is very desirable to have two sensitive urinometers, one registering from 1.000 to 1.025, and the other from 1.025 to 1.050, and reading to four places of decimals.

A urine glass is filled with the fresh urine (about 4 oz.), and the specific gravity carefully ascertained, the temperature of the urine being noted at the same time. A piece of fresh good yeast as large as a small walnut is next added to the urine, the glass gently shaken, and then placed in a warm part of the room, covered with filter-paper and a small tumbler or beaker. The urine must be left at least twenty-four hours, sometimes longer, till all fermentation is accomplished. The specific gravity is now taken again with due care, and at the same temperature as before, which can be accomplished by placing the urine jar in a larger vessel of water, to which cold or hot may be added as required. The specific gravity

of the urine is read off, and subtracted from that obtained before fermentation. It has been found as a result of comparison between samples of saccharine urine tested in this way, and by other methods, that a difference of 0.001 or one urinometer degree in specific gravity corresponds to 0.230 grms. of sugar per cent. or one grain of sugar per ounce, so that a calculation can easily be made.

Ex. Specific gravity before fermentation = 1038.

Specific gravity after fermentation = 1010.

Difference = 28.

$28 \times 0.230 = 6.44$ per cent.

And $6.44 \times 4.375 = 28.1$ grs. per ounce.

If desired, a second sample of the same urine, but without the addition of yeast, may be kept under similar conditions to that which is fermenting; the specific gravity of each may then be taken at the end, and the difference noted.

(3) *The Polariscopes.* This instrument may be used to estimate the amount of glucose in a given specimen, from its dextro-rotatory effect on a plane of polarized light. As albumin is laevo-rotatory it must first be removed if present, and if the urine is very dark in colour it should be filtered through animal charcoal to decolorize it.

The instrument may be provided with a scale, indicating without the need of further calculation the percentage of dextrose present, and so in this way one can perform an estimation both quickly and surely.

One drawback is the cost of the instrument, and as it is seldom used in ordinary clinical work, more detailed information as to its working will not be given here.

(4) A fourth instrument which is not very largely used is Johnson's *Picro-saccharimeter*, where an estimate is made by contrasting the tint obtained in performing the picric acid test, with a standard red solution of acetate of iron.

Lactose or Milk-sugar ($C_{12}H_{22}O_{11}$). Not infrequently one finds sugar in the urine of women who are suckling their children, the result of the passage of lactose direct from the mammary gland into the blood, and thence to the kidneys.

Milk-sugar in urine reacts to Trommer's, Fehling's, and Böttger's tests, but does not yield an osazone with phenylhydrazine as a rule, though a pure solution of lactose will. Under the influence of yeast it is slowly inverted into dextrose and galactose and then fermented.

Sugar of milk does not reduce a cupric salt so actively as grape-sugar; if, for example, 7 grs. of grape-sugar reduce a given amount of Fehling's solution, it will require 10 grs. of lactose to produce the same effect.

Laevulose or Fruit-sugar ($C_6H_{12}O_6$). This body, as already stated, may occur in urine, and gives exactly the same tests as glucose; it fails, however, to rotate the plane of polarized light to the right.

Pentoses ($C_5H_{10}O_5$). In addition to the sugars already described, there may occasionally be found small quantities of five carbon sugars called pentoses, examples of which are rhamnose and arabinose. They seem in some instances to be derived from fruits, such as plums and cherries, and in others to arise in the course of various diseases, without, however, being of any special significance.

Tests for pentoses. They reduce Fehling's and Trommer's solutions, but do not ferment with yeast; they yield a

pentosazone with phenyl-hydrazine. They also exhibit what is known as "Tollen's reaction," and which is elicited by dissolving a little phloroglucin in 5 c.cm. of hydrochloric acid in a test-tube, adding 5 c.cm. of the urine, and then holding the tube in a small beaker of boiling water for a few minutes. In the presence of pentoses a red froth is developed, and the colour goes down through the fluid. Glycuronic acid yields a similar reaction.

Iso-maltose. A minute trace of this carbohydrate has been found in the urine, even in health, but is of no special importance.

Glycuronic acid ($C_6H_{10}O_7$). This substance not infrequently appears in urine under special conditions, and merits a short description. Its constitutional formula is $CO_2H.(CH.OH)_4COH$, from which it is seen to be of the nature both of an aldehyde and an acid, and to be closely related to glucose ($CH_2OH(CH.OH)_4COH$). It appears to arise in the course of the metabolism of sugars within the body, but as the metabolic changes usually go on much further than the stage at which it is formed, it does not appear in the urine. It occasionally happens, however, that a patient is taking some special article of diet or medicine which will yield something that can join itself to the glycuronic acid in the system, and in this way that substance may come to find itself in the urine. This especially happens after the use of such drugs as chloral, butyl-chloral, and chloroform, and it is important that this point be kept in mind when examining the urine of a patient who has been taking these medicines.

Tests. (1) It reduces Fehling's and Nylander's solutions,

and this fact constitutes its chief claim for consideration ; otherwise it is of little importance.

(2) It gives Tollen's reaction (*vide supra*), with phloroglucin and hydrochloric acid ; and

(3) It does not ferment with yeast.

Acetone (CH_3COCH_3). When this body occurs in urine, we deal with what is termed "acetonuria," which is most frequently met with in diabetes mellitus. Acetone itself is a colourless liquid with a pleasant fruity smell, which may be communicated to the urine when it is present in it. In addition to diabetes, it is found in the urine not infrequently in fevers, in cancer, and in digestive disturbances. Its presence certainly seems to be associated with an increased metabolism of albuminous tissues. As regards its significance, its presence in cases of diabetes usually indicates that the disease has assumed a grave form, and it is generally regarded as an unfavourable sign. Von Jaksch says it may occur in perfect health (physiological acetonuria).

Tests for acetone. In some cases the urine may be tested directly, but if the quantity of acetone be small, it is better to distil it off from say 500 c.cm. of urine, to which a little phosphoric acid has been added.

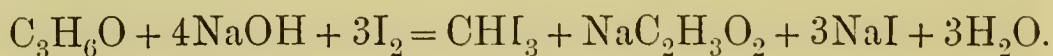
(1) *Legal's test.* To 5 c.cm. of urine (or distillate) add three drops of a freshly-prepared fairly strong solution of sodium nitro-prusside, and then enough solution of caustic soda to make it strongly alkaline. A red colour is immediately struck, which speedily turns to yellow. All urines, however, show this (see creatinin, p. 40), which is not the essential part of the test. But if acetic acid is added just as the colour is changing from red to yellow, a fine reddish-

purple colour appears in the presence of acetone, and it is this that is indicative of the foreign element. This is not a very reliable test, as paracresol gives the same reaction, and error will occur more readily if the urine be distilled as the paracresol passes over too.

(2) *Gunning's test.* This is good and sufficiently delicate, and is said to be beset by no fallacies (Sahli). It is performed on the urine or distillate by adding to 10 c.cm. in a test-tube a small quantity of tincture of iodine (B.P.), and then enough solution of ammonia to produce a dark black precipitate of iodide of nitrogen.

If acetone be present, this precipitate, after standing a short time, will become yellow from the formation of iodoform, which can be readily recognized by its smell, and by the form of its crystals (hexagons and stars) as seen under the microscope.¹

(3) *Lieben's test.* This is the most sensitive test of all, and it also depends on the formation of iodoform. It is performed by taking 5 c.cm. of the distillate in a test-tube, adding five drops of a 10 per cent. solution of caustic soda, and warming gently. There is then added, drop by drop, a saturated solution of iodine in iodide of potassium (aqueous solution) till a permanent yellow tint is got. More of the caustic soda solution is now added, till the yellow colour is discharged; and very speedily, if acetone be present, a precipitate of iodoform will occur, thus:



¹ The author has found that a scanty formation of iodoform may occur from liquor ammoniae and tincture of iodine even in the absence of acetone, so that this test is not absolutely reliable.

Alcohol may give this reaction, so the urine used must be fresh.

Aceto-acetic or di-acetic acid ($\text{CH}_3\text{COCH}_2\text{CO}_2\text{H}$). This body is acetic acid where an atom of hydrogen has been replaced by the radical acetyl, CH_3CO , and is the same as the molecule of acetone, less CO_2 . It only occurs in urine under pathological conditions. Acetone is almost always found as well when it is present, and it is to be regarded as of serious significance. It occurs in grave cases of diabetes, especially those tending to coma; it may also occur in severe fevers.

Tests. As this body is somewhat volatile and not very stable in urine, the specimen taken for examination must be freshly passed. To 10 c.cm. add drop by drop a weak solution of perchloride of iron, which in some cases may at once give a bordeaux-red colour (due to the di-acetic acid), but may in others produce at first a precipitate of phosphate of iron. If this be filtered off, and more iron added to the filtrate, the red colour will appear in the presence of the acid. This test, however, as it stands, is not sufficient, as acetates, salicylic acid, etc., may also strike red with perchloride of iron. We may make sure of the di-acetic acid in one of two ways:

(1) Since it is volatile it can be driven off by heat; we therefore take a second sample and boil it before adding any iron solution. If on testing now we get no red coloration, we know that di-acetic acid was present in the first sample; if the colour be fainter, but still present, we know that it was in part due to this acid.

(2) Or we may take 10 c.cm. of urine, add a few drops of sulphuric acid, 3 c.cm. of ether, and shake; the latter

fluid can dissolve any di-acetic acid that is present. After the ether has risen to the top it may be pipetted off, shaken up with dilute solution of perchloride of iron, when, if the acid be present, the watery layer will become claret-coloured.

Oxy-butyric acid ($\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{CO}_2\text{H}$). This acid occurs along with acetone and di-acetic acid in grave cases of diabetes, and need have no special tests applied to it; it is a laevo-rotatory body, and so diminishes the dextro-rotatory power of glucose as shown by the polariscope. On distillation it yields crotonic acid ($\text{C}_3\text{H}_5\text{CO}_2\text{H}$).

CHAPTER VI.

*Pathological elements in urine continued: Blood-pigments—
Haematoporphyrin — Bile-pigments — Bile-salts— Urobilin—
Melanin—Hydrochinon—Alcapton.*

Blood-pigments. In studying the naked-eye appearances of urine it has already been pointed out that the colour may be altered from the presence of blood. It is, however necessary to have more exact methods of identifying the foreign element than merely by the eye, and we must therefore consider what chemical tests can be applied. Of course in all cases the microscopic appearances must be carefully looked to.

The pigment that is most commonly present is oxy-haemoglobin; but if the urine has held the blood long, and in cases of haemoglobinuria, methaemoglobin may be present, giving a brownish tint, rather than a red one, to the urine.

Blood in the urine, or haematuria, occurs in various diseases, medical and surgical, of which the chief are acute nephritis, tumours, especially cancer of the genito-urinary tract, calculus of the kidney and bladder; also in general diseases such as scorbutus, purpura and others; after the use of some poisonous or irritating drugs, such as can-

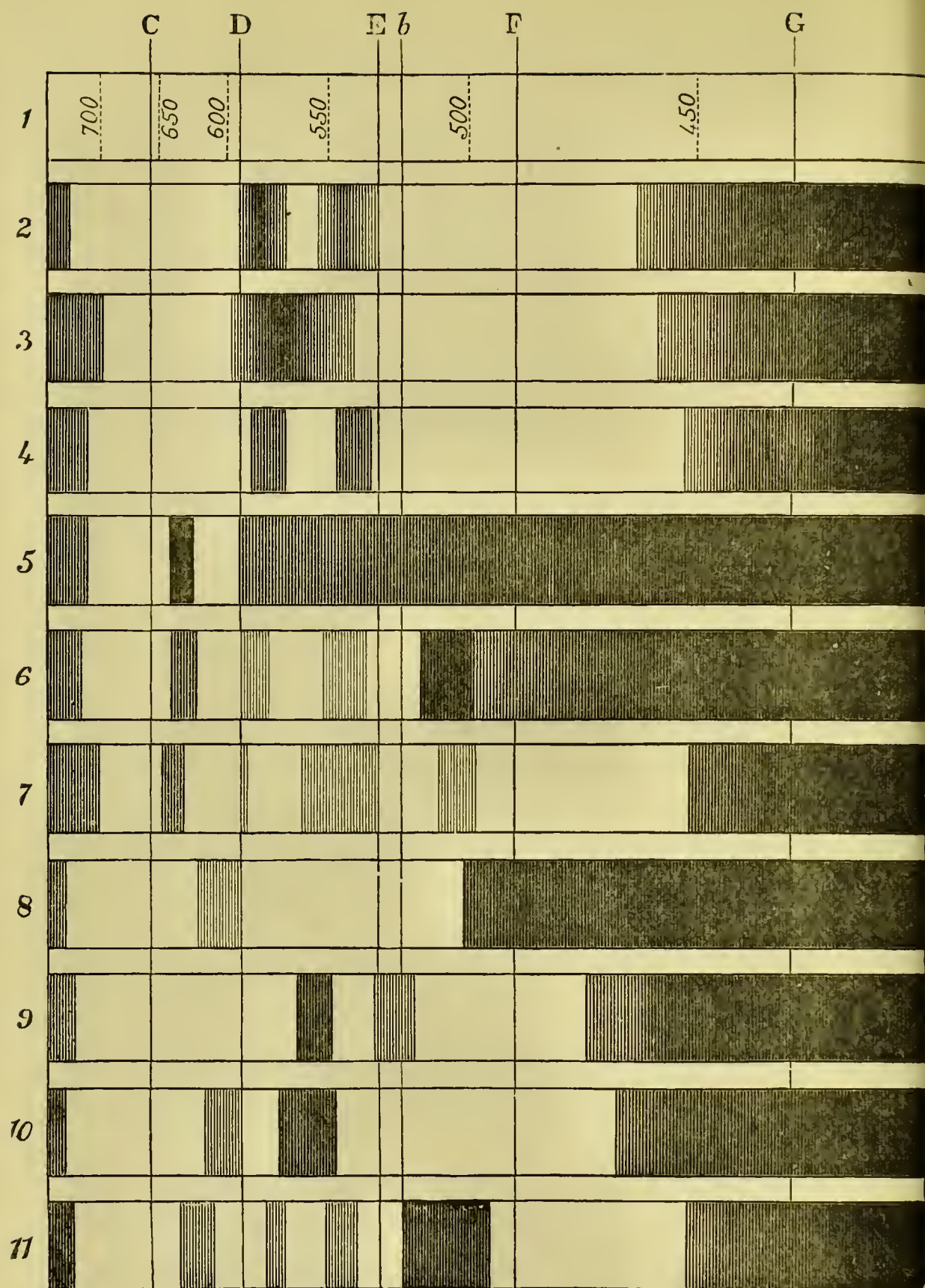


FIG. 8.—TABLE OF SPECTRA. 1. Solar spectrum; 2, spectrum of oxyhaemoglobin; 3, spectrum of haemoglobin; 4, spectrum of CO-haemoglobin; 5, spectrum of met-haemoglobin (concentrated solution); 6, spectrum of methaemoglobin (dilute solution); 7, spectrum of acid haematin; 8, spectrum of alkaline haematin; 9, spectrum of haemochromogen (reduced haematin); 10, spectrum of acid haematoporphyrin; 11, spectrum of alkaline haematoporphyrin. (Halliburton.)

tharides and turpentine, and in mechanical injuries to the kidney, ureter, bladder, prostate, or urethra. Blood may also be due to the presence of parasites such as the *Distoma haematobium*.

The higher up the source of bleeding is, the more intimately will the blood be mixed with the urine, and the less readily will it form a sediment.

In nearly all cases of haematuria the microscope will reveal the presence of red blood cells, but in some cases of poisoning by cantharides, creasote and turpentine, in the disease known as idiopathic or paroxysmal haemoglobinuria, and in some cases of malaria and severe burns, the urine, though it contain blood-pigment, reveals under the microscope no corpuscles, but only small particles of brown pigment. The pigment in such cases is frequently in the form of methaemoglobin, which can really only be distinguished from oxy-haemoglobin by the spectroscope, the former showing four absorption bands, while the latter exhibits two, becoming one (reduced haemoglobin) on the addition of ammonium sulphide (see fig. 8).

In using the spectroscope clinically, a small direct-vision pocket instrument will be found quite suitable for all ordinary purposes. If desired it may be mounted on a simple stand. It should first be focussed by moving the eye-piece till the dark Fraunhofer's lines are seen, and then by narrowing the slit the lines should be rendered as sharp as possible. Observations are made in daylight, and the fluid to be examined is placed in a test-tube held an inch from the spectroscope. Garrod recommends the use of a conical glass, holding say six ounces, as then varying thicknesses of fluid can be examined without

pouring from one glass to another. (For table of spectra see fig. 8.)

Chemical tests for blood. (1) *Heller's test.* To 5 c.cm. of urine add 10 drops of caustic potash solution, and boil; the earthy phosphates are precipitated, and as they carry down with them the haematin, which results from the splitting up of the haemoglobin, they become coloured red or brownish-red. The deposit may not be very obvious at first, but becomes more distinct after the tube has stood for a short time. Rhubarb, senna, and santonin, given by the mouth and excreted in the urine, may also yield a red precipitate on boiling with an alkali, but can be distinguished from blood by the next test.

(2) *Guaiac and ozonic ether test (Almén's).* In performing this test 5 c.cm. of urine are placed in a test-tube, and to this are added two or three drops of fresh tincture of guaiac, and then 2-3 c.cm. of ozonic ether or of old (*i.e.* well-oxidized) oil of turpentine, and the whole gently shaken. When the guaiac is added a whitish precipitate appears in every case owing to the resin being thrown out of solution, but this clears up a little on adding the ether, and, if blood be present, a blue line appears at the junction of the urine and the lighter ether which floats upon it. Where the blood is abundant quite a broad band of blue appears. Ozonic ether consists of a solution of peroxide of hydrogen in ether, and the oxygen which this yields is taken up by the haemoglobin and handed on to the guaiac, which then assumes a blue colour.

Some advise that the ozonic ether and tincture of guaiac should be mixed together first in equal parts, and then poured on to the top of the urine in a test-tube,

but this method possesses no advantages over that described above. This test is a delicate one, but certain points must be observed in its performance. In the first place, the reagents must be reliable; the guaiac tincture must be fresh and preserved in a dark glass bottle; if ozonic ether is used, it also must be fresh, but if oil of turpentine is employed, it acts better when old. Secondly, we must be sure that the sample of urine taken contains a little of the sediment, if any has fallen, as most of the blood may have collected at the bottom of the urine-glass. Thirdly, pus in urine may give a closely similar reaction, but it does not react to Heller's test, and the microscope reveals the pus cells. Further, if the patient be taking preparations of iodine, a bluish-green colour may appear. Any doubt on this point may be quickly cleared up by taking a second sample of the urine, boiling it with a fragment of starch, and, when cool, adding a little strong nitric acid. The latter liberates the iodine, which strikes a dark blue colour with the starch. The addition of nitric acid discharges the blue colour due to blood, while in that arising from iodides it causes the blue to disappear and replaces it by a yellow tint from the liberated iodine.

(3) *Test by formation of haemin crystals.* This may be performed with the sediment from Heller's test collected and dried, or, as Struve advises, the urine may be treated with caustic potash and then with acetic and tannic acid, when a dark precipitate falls, which also may be collected and dried. In either case a little of the dried sediment is placed on a glass slide, a few small particles of sodium chloride added, and a cover-

glass put on. A full drop of glacial acetic acid is next allowed to run under the cover-glass, and the slide is



FIG. 9.—HAEMIN CRYSTALS. (Halliburton.)

gently warmed over a spirit lamp for a minute or two. The acetic acid will boil away quietly, and a little more should be added from time to time. Over-heating must be avoided as it spoils the preparation.

If the specimen be examined microscopically when cool, small mahogany-brown rhombic crystals (see fig. 9) of haemin will be found, often called Teichmann's crystals. Haemin is the hydrochloride of haematin, the acid being derived from the sodium chloride and acetic acid. This test is very delicate, but not always easy to carry out.

Haematoporphyrin. This compound, which is iron-free haematin, is derived from haemoglobin in the body, and in some instances, perhaps, arises also through disordered chemical processes in the metabolism of blood-pigment, whereby it is produced in place of bilirubin.

Haematoporphyrin exists in small traces in healthy urine, possibly as a chromogen, and constitutes one of the normal pigments. It is, however, present in scanty amount, and if it respond to any ordinary test, it is safe to infer that it is increased in quantity.

Urines which exhibit "haematoporphyrinuria" are of a port-wine colour, sometimes even darker, with a tendency

to opacity. The colour is not altered on boiling. Haematoporphyrin has been found in the urine on many occasions after prolonged use of sulphonal, and, to a less degree, of trional; its presence is a warning that the drug should be stopped at once. It has also been found in cases of Addison's disease, enteric fever, cancer and cirrhosis of the liver, and lead-poisoning.

Spectroscopically, haematoporphyrin shows four absorption bands in alkaline solution, and two in acid (fig. 8).

Chemical test for haematoporphyrin (Riva and Zoja's). Take 50 c.cm. of the suspected urine, and shake up thoroughly but gently with 10 c.cm. of amylic alcohol in a cylindrical glass. After the mixture has been allowed to stand for a little, the amylic alcohol will rise to the top, and may be removed by a pipette. To this alcoholic extract a little ammoniacal 5 per cent. solution of zinc chloride in absolute alcohol is added, when a red precipitate will form if haematoporphyrin is present. (Any green fluorescence that may appear is due to urobilin.) If a little hydrochloric acid be now added to the amylic extract it will take up the red colour and assume a violet-red tint.

Bile-pigments. Of the various pigments that exist in bile, v. Jaksch asserts that only bilirubin occurs in urine freshly passed, and that if others are found they are its oxidation products. Bile in urine produces the condition known as "choluria," and is a well-known indication of jaundice, particularly in those cases where, from any cause whatever, be it calculus, tumour, or simple catarrh, the bile duct becomes occluded or blocked. Cases of this kind are often spoken of as "hepatogenous" jaundice, indicating that the pigment is derived from the liver.

In other cases, however, there may seem to be no affection of that organ whatever, and since it would then appear that the pigment arises from blood-colouring matter outside the liver, the condition is named "haematogenous" jaundice. The latter fact warns us not to conclude that we are dealing with a case of liver disease, merely because bile appears in the urine. The observer's attention may be directed towards the possibility of bile in urine by the obvious change in colour of that fluid from an amber tint to a greenish-brown; when the urine is shaken it presents a yellow-tinged froth. The quantity of bile present, however, may be so scanty that the urine merely appears of a golden hue. There are several chemical tests for bile-pigment in urine, of which three will be given.

(1) *Gmelin's test.* This may be performed in several ways. We may spread out a little of the urine on a white plate, and drop into the centre of the film a little nitric acid containing nitrous; in the presence of bile-pigments, under the oxidizing power of the acid, a play of colours is produced in the form of concentric rings showing red, green, blue, and yellow, the green being the most distinct. Or a little of the acid may be placed in a conical glass and the urine run on to the top of it, when the colours will become visible at the junction of the two liquids. Or, finally, the modification of the test suggested by Rosenbach may be used where the bilious urine is filtered, and the acid applied to the moist yellow-stained filter paper.

(2) *Iodine test (Maréchal's).* To perform this test, place 5 c.cm. of urine in a test-tube, diluting it with a little water if the pigment be very plentiful. On to the surface

of the urine a small quantity of weak tincture of iodine is now run, and at the junction of the fluids a fine green ring, coloured with biliverdin, will appear. The iodine solution may be made of the *tinctura iodi* B.P., diluted with twice its volume of rectified spirits; it floats on the urine on account of its lower density. If the green ring does not show at once, the tube may be shaken gently to bring more urine in contact with the iodine.

(3) *Huppert's test*. This is very delicate, and may be employed where only small traces of pigment are present. To perform it, place 10-15 c.cm. of the urine in a small beaker, and add milk of lime, which will throw down a precipitate of phosphates, sulphates, etc., carrying with it the bile-pigment which stains the sediment yellow. The mixture is filtered and a little of the precipitate removed from the filter paper to a test-tube, and treated with sulphuric acid, till acid, and absolute alcohol. If it be now boiled the sediment loses its colour, while the clear liquid assumes a distinct green tint, easily seen against a white background.

Bile-salts. There appears to be little reason to doubt that these, as well as the bile-pigments, occur in the urine in jaundice, but their amount is so small, and the tests for them in urine so doubtful, that clinical investigations upon them are not often made, and appear indeed to be of little value.

There are several tests described, of which probably the best known and least reliable is that associated with the name of Pettenkofer. It is usually recommended that it be performed by shaking up a little urine with some syrup of cane-sugar (20 per cent.) in a test-tube, and

allowing a drop or two of strong sulphuric acid to trickle through the froth, when the appearance of a red colour denotes the presence of bile-acids. But so many other substances may produce the same effect that the test carried out in this way is of little value.

Peptone test. Oliver has suggested the use of papers soaked in peptone as a reliable test for bile-acids in urine. The papers are supplied along with a set impregnated with citric acid, and are used as follows: The urine is first filtered and reduced by adding water to a specific gravity of 1008. One peptone paper and half a citric acid one are placed in 4 c.cm. (60 m.) of water in a test-tube, gently shaken, and after a minute or two withdrawn. Twenty minims of the urine, prepared as above, are now added, the tube shaken and set aside for a minute, when an excess of bile-salts will be indicated by the appearance of a distinct milkiness.

As a rule, however, bile-salts cannot be demonstrated in urine but must first be isolated, a procedure not suited as a rule for ordinary clinical work, although the following method may be carried out without much trouble.

Dragendorff's method of isolating bile-salts. 150 c.cm. of urine, previously acidulated with a little hydrochloric acid, are shaken up with 30 c.cm. of chloroform for an hour. On standing, the chloroform sinks and the urine may be decanted off. To the chloroform extract are now added 8 c.cm. of absolute alcohol and the mixture is filtered, after shaking. The filtrate, composed of alcohol and chloroform, is allowed to settle, when the lighter alcohol rises to the top and may be pipetted off. It is it which contains the bile-salts, and it must now be

evaporated to dryness. A little of the dry residue is dissolved in say 10 c.cm. of water, and 6 c.cm. of strong sulphuric acid added slowly, so that the temperature may not rise above 60° C. Four drops of a 20 per cent. solution of cane-sugar are added and the whole shaken, when a violet colour is developed if bile-salts are present.

Urobilin. This interesting body forms, in minute amount, one of the normal pigments of urine present as a chromogen when the urine is first passed. But if it can be demonstrated with any degree of ease at all, it is to be regarded as a pathological element. Macmunn, who has contributed largely to our knowledge of the pigments in urine, thinks that normal and pathological urobilin are different, but Hopkins and Garrod¹ have recently given reasons for holding that they are identical. Urobilin is closely allied to, if not identical with, the substance stercobilin found in the faeces, and in most cases both these bodies appear to be derived from the bilirubin of the bile by changes in the intestinal canal, possibly under the influence of micro-organisms. But urobilin certainly appears to be produced directly from disintegrated red-blood cells in cases of extravasation of blood into various tissues. Urobilin itself is an amorphous brown pigment, soluble in alcohol but not in water. Urine which contains it in large amount is of a brown-red colour.

This pigment occurs in pernicious anaemia, Addison's disease, scurvy, fevers, and in effusions of blood, *e.g.* intracranial haemorrhages and uterine haematocele.

The spectrum of urobilin in an acid solution shows one distinct band between the green and the blue.

¹ *Journal of Physiology*, vol. xx., 1896.

Chemical tests. Urobilin is easily demonstrated owing to the fact that it causes a fine green fluorescence with chloride of zinc, forming a compound with the zinc. If it is abundant the test can be applied direct to the urine by adding a few drops of a 5 per cent. solution of zinc chloride in absolute alcohol and then liquor ammoniae till the precipitate formed is dissolved. The presence of urobilin is made apparent by the urine exhibiting a green fluorescence. If the quantity present is small, the urine must first be acidulated with a few drops of hydrochloric acid, and then shaken up gently with one-third its volume of amylic alcohol, which will extract the urobilin. After an hour the alcohol may be removed with a pipette and treated as above with zinc chloride and ammonia, when it will show a fine green fluorescence. Should a red precipitate form in the amylic extract, this indicates the presence of haemato-porphyrin (see p. 101).

Melanin. This colouring matter may be found in various morbid conditions, and as it exists at first as a chromogen, the urine when passed does not look abnormal but darkens after a time, from oxidation. Artificial oxidizing agents produce the same effect but more quickly.

The presence of melanin in urine has been regarded as an important sign in cases of melanotic sarcoma and cancer, but it certainly appears as often in other cases of wasting disease.

(1) *Zeller's test.* To 10 c.cm. of urine add drop by drop fresh bromine water, when a yellow precipitate falls, becoming black on standing.

(2) *Ferric chloride test.* To 10 c.cm. of urine add drop

by drop a fairly strong solution of perchloride of iron, when a precipitate of the phosphates occurs, carrying down a grey pigment if melanin be present. Melanin may resemble indigo-blue in appearance, but differs from it in not being soluble in ether.

Hydrochinon. This substance, which chemically is para-di-hydroxy-benzene ($\text{C}_6\text{H}_4\begin{smallmatrix} \text{OH} \\ \text{OHp.} \end{smallmatrix}$), is present in the urine in cases of carbohic acid poisoning, and is the chief agent which causes the urine to assume a dark colour. Hydrochinon is not excreted as such, but is combined with sulphuric acid as a conjugated sulphate. There is no simple chemical test that can be applied for the certain detection of hydrochinon. If a little of the urine containing it be boiled with a few drops of hydrochloric acid it becomes an active reducing agent, and can cause a deposit of metallic silver from an ammoniacal solution of silver nitrate.

Alcapton. Occasionally we meet with a condition of the urine termed "alcaptonuria," where it becomes very dark after exposure to the air. The substance supposed to cause this dark coloration has been named alcapton, and is not the same body in all cases. In a certain number it appears, from the investigations of Baumann and Wolkow,¹ to be homogentisinic acid, but in others it is some complex acid of the aromatic series. In all cases it appears to be derived from some perverted metabolism within the body, and is occasionally seen in diabetes and phthisis. It is of little real importance, but merits a passing notice owing to the fact that urine containing it can reduce Fehling's solution.

¹ *Zeitschr. für physiol. Chemie*, Bd. xv., 1891, s. 228.

CHAPTER VII.

Pathological elements in urine continued: Leucin—Tyrosin—Cystin—Sulphuretted hydrogen and other gases—Alkaloids and ptomaines—Diazo-reaction—Chyluria—Bacteriuria—Toxicity of urine—Drugs in urine—Antipyrin—Phenacetin—Salicylic acid—Carbolic acid—Tannic acid—Bromides—Iodides—Rhubarb—Senna—Chrysophanic acid—Santonin—Vegetable alkaloids—Copaiba.

Leucin and Tyrosin. These two bodies may be conveniently taken together, as they occur in association in the urine in various diseases.

Leucin ($\text{C}_6\text{H}_{11}\text{NH}_2\text{O}_2$) was formerly considered to be amido-caproic acid, but now appears to be α -amido-isobutyl-acetic acid, an isomer of the acid first named. It is fairly soluble in water, and occurs along with tyrosin in cases of acute yellow atrophy of the liver, and, less frequently, in phosphorus poisoning. Its appearance is thus associated with disorganisation of the liver.

The student is often led to expect that it can readily be found in the sediment of urines that contain it, but this is quite a mistake, as it rarely occurs as a deposit without special treatment. It may, however, be obtained by concentrating the urine by evaporation and extract-

ing the residue with hot absolute alcohol and filtering, when, as the filtrate cools, a deposit of leucin will occur, consisting of very small spheres with concentric markings (fig. 10, c).

Chemical test for leucin. Dissolve some of the above deposit in boiling water, and add a little of a 10 per cent. boiling solution of cupric acetate, when a dark-blue crystalline compound will form.

Tyrosin ($C_3H_4(C_6H_4.OH)NH_2.O_2$) is para-oxyphenyl-amido-propionic acid, and is also partially soluble in

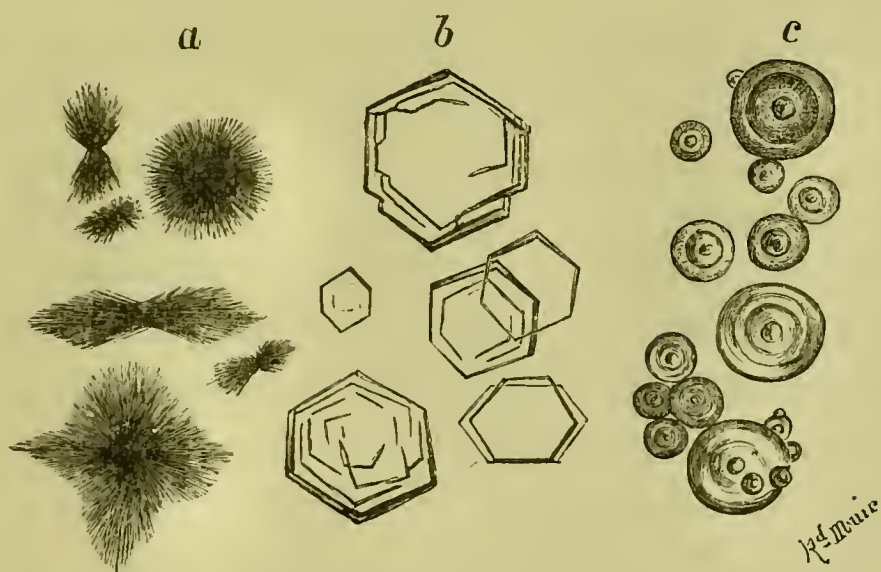


FIG. 10.—a, TYROSIN ; b, CYSTIN ; c, LEUCIN. (Hutchison and Rainy.)

water. It too, very seldom occurs as a deposit in urine. To obtain it the urine should be treated with basic acetate of lead, which will precipitate many things, but not the tyrosin. On filtering, the filtrate must be treated with a stream of sulphuretted hydrogen to get rid of the lead it contains, and again filtered. The filtrate which contains no lead is now evaporated to a small bulk, and, as it cools tyrosin separates out in crystals,

which are in the form of fine needles arranged in sheaves or clusters (fig. 10, *a*).

Scherer's test for tyrosin. A little tyrosin evaporated with a drop or two of strong nitric acid on a piece of platinum foil leaves an orange-yellow residue. This if moistened with caustic soda solution becomes red.

Cystin ($C_6H_{12}N_2S_2O_4$). This body occurs but rarely in urine, and gives rise to the condition termed "cystinuria." As its formula shows, it contains sulphur.

Cystinuria is an infrequent condition, and is sometimes hereditary. It was in the urine of a cystinuric patient that Udránsky discovered bodies named "diamines," examples of which are tetra- and penta-methylen-diamine, which were proved to be identical with the ptomaines cadaverin and putrescin found in putrid flesh by Brieger. This is a good example of auto-intoxication by absorption from the intestinal canal, where such bodies originate. Brieger and Stadthagen¹ thought that the diamines acted as bases to the cystin, carrying it as a salt into the bladder, where it is decomposed. Cystin crystallizes very definitely in flat hexagonal crystals (fig. 10, *b*) insoluble in acetic acid, but soluble in mineral acids and in alkalies; it may occasionally form a calculus.

Chemical test. A little of the urine containing it is heated with caustic potash and acetate of lead, when the sulphur in the cystin goes to form a sulphide of potassium which gives a black precipitate with the lead salt.

Sulphuretted hydrogen. This has been found in urine, and such a condition is described by the somewhat clumsy name, "hydrothionuria." The gas is usually formed by

¹ *Berlin. klin. Wochens.*, 1889, s. 344.

the action of the bacillus coli communis on substances in urine, such as cystin, which contain sulphur.

Unless it be present in freshly passed urine, we cannot say we are dealing with a true case of hydrothionuria as the gas may readily develop in old urine outside the body. The condition, when it is present, occurs in cystitis and pyelitis; there is little evidence to show that sulphuretted hydrogen developed in the intestine ever passes through the blood to the urine. It might of course pass directly in cases of fistula between the bowel and ureter or bladder.

Test for sulphuretted hydrogen. Advantage may be taken of the effect of the gas on lead-salts to demonstrate its presence. About 150 c.cm. of the urine plus a little hydrochloric acid are placed in a flask, and a tightly fitting cork inserted, to the under surface of which is attached a strip of filter-paper soaked in solution of plumbic acetate and caustic soda. The flask may be shaken from time to time, and the presence of the gas will be indicated by the blackening of the test-paper.

Other gases in urine. The distinct presence of other gases in fresh urine has been termed "pneumaturia." It is found in some cases where the bacillus coli is present in the bladder. Cases have been reported where alcoholic fermentation occurred in diabetic urine with the evolution of carbonic dioxide, and v. Frerichs¹ has recently published notes of a case where the urine bubbled as it was drawn off, and contained white granular bodies of the nature of thrush. The gases present are usually carbonic dioxide and hydrogen.

¹ *Wien. klin. Wochen.*, No. 29, 1898.

Ptomaines and alkaloids in urine. In some cases of disease, toxic bodies of alkaloidal nature have been discovered in the urine. It has already been pointed out that cadaverin and putrescin have been found in the urine of cystinuric patients. Albu¹ investigated various cases of scarlet fever, measles, diphtheria, erysipelas, etc., and discovered, out of twenty-three experiments, a toxic principle in the urine in fourteen, and Ewald and Jacobson² obtained ptomaines in the urine in cases of severe gastric disorder.

As yet, however, little information of any real and positive value, either as regards diagnosis or prognosis, has been acquired, and the processes involved in carrying out such researches require the resources of a fully equipped laboratory.

The diazo-reaction. This reaction, introduced by Ehrlich as a diagnostic sign in typhoid fever, has been relegated to an unimportant position, both on account of its doubtful reliability and because Widal's serum test has attracted general attention. It may be well, however, to describe it briefly. Ehrlich found that diazo-benzene-sulphonic acid produced a red colour in the urine in many cases of typhoid fever and measles, and it is this point on which the test is based. The diazo-benzene-sulphonic acid is not kept ready made, but is developed as required from acid solutions of sulphanilic acid and sodium nitrite.

Two test-solutions are requisite: (1) A 5 per cent. solution of hydrochloric acid in water, saturated with sulphanilic acid.

Berl. klin. Wochens., Nos. 1 and 48, 1894.

² *Ibid.*, No. 2, 1894.

(2) A 0·5 per cent. solution of sodium nitrite in water.

To perform the test 40 c.cm. of the first solution should be mixed with 1 c.cm. of the second, the mixture then added to an equal volume of the urine in a conical glass, and the whole shaken. A little liquor ammoniae must now be run down the side of the test-glass, when a distinct red ring or coloration forms if the reaction be positive. If the solution remain yellow or orange, it is negative. v. Jaksch considers that no reliance should be placed on it at all, and that the positive reaction simply indicates acetone.

Chyluria. The presence of chyle in the urine has already been referred to on p. 5, under alterations in colour of that fluid. In this condition there are found fat, albumin, and fibrin, the latter tending to make the urine gelatinize on standing. It is caused by a parasite, the *filaria sanguinis hominis*, which probably leads to the formation of abnormal communications between the lymph channels and the ureters or bladder.

Bacteriuria. This name is applied to cases where bacteria, micrococci, fungi and moulds, appear in the urine as it is passed. In some instances the organisms present are non-pathogenic, such as yeast-cells, moulds, and even sarcinae. In other cases pathogenic organisms are found, notably tubercle-bacilli, and to a less degree other germs such as gonococci, micrococci in erysipelas, and the actinomyces. Houston¹ has recently reported a very interesting case of cystitis due to the typhoid bacillus of Eberth, but where no typhoid fever occurred. The name "idiopathic bacteriuria" is sometimes applied to cases where the

¹ *Brit. Med. Jour.*, vol. i. p. 78, 1899.

presence of organisms (usually non-pathogenic) is the only obvious morbid sign. Such cases require bacteriological rather than chemical investigation.

To examine the urine for tubercle bacilli, the centrifuge should be used to obtain a sediment. Very commonly this consists largely of pus, and often of urates or phosphates. Urates can be dissolved by placing the tube containing the sediment in a beaker of warm water. As much of the liquid having been poured off as possible, cover-glass preparations are made with the sediment, allowed to dry in the air, and then stained by the Ziehl-Neelsen method (see sect. iii.). The bacilli, about one-third the diameter of a red blood-cell in length, may occur singly or in clumps. Several films should be carefully examined.

The smegma-bacillus, which exists in the secretion under the foreskin in men, and between the labia majora in women, closely resembles the tubercle bacillus, and stains with fuchsin, which is not removed by mineral acids, owing, in the opinion of Flügge,¹ to the fatty substratum in the organism. Decolorization by mineral acids can be effected however if the films are first soaked for a few minutes in a weak warm alcoholic solution of caustic alkali (which probably saponifies the fatty substance), and then washed in alcohol. Flügge suggests that if doubt exist, inoculation experiments should be tried, as the smegma bacillus is not easily conveyed to lower animals.

The gonococcus of Neisser usually occurs in pairs, each organism being of the shape of a kidney or bean, and lying with the concave sides facing one another. They

¹ Flügge, *Die Mikro-organismen*, p. 517, 1896.

are small in size, and are usually found in the pus-cells in cases of gonorrhoea. Films may be prepared on cover-glasses, dried in the air and fixed by heat, and then stained with a watery solution of some basic aniline dye, such as fuchsin. The gonococcus is decolorized by Gram's method.

Pyuria, or the presence of pus in the urine, will be discussed in detail when treating of the urinary sediments (see p. 126).

Toxity of the urine. Of late years much attention has been directed to the toxic properties of the urine as a whole, and their variation under different conditions of health and disease. Bouchard, Charrin, and Roger in France, have paid special attention to this point, and have elaborated it to an extraordinary degree, especially with reference to auto-intoxication. In order to compare results it is necessary to have a standard, and this is supplied by the "urotoxic co-efficient" of Bouchard, which is indicated by the weight in kilogrammes of an experimental animal (say a guinea-pig), which can be killed by the urine secreted in twenty-four hours by one kilogramme of the person under consideration.

For example, as one kilogramme of body-weight of a healthy man can secrete enough urine in twenty-four hours to kill 0.461 kilog. of an animal experimented on, the human urotoxic co-efficient in health is set down as 0.461. The urine in such experiments is introduced into a vein of the animal.

The toxicity of the urine is not constant; it is diminished notably under a milk diet, and also in old age, in pregnancy, when intestinal antiseptics are used, and in

some cases of eclampsia (because the poisons are retained in the body). It is said to be increased in the cachexia of cancer, in acute infectious diseases, in many affections of the liver, and after the crisis of pneumonia. Many items of interest in connection with this subject will be found in Bouchard's¹ writings.

Drugs in urine. In most cases drugs administered by the mouth find their way out of the system by way of the kidneys, and often in a more or less altered form. Advantage may be taken of this to discover whether or not a patient is taking a prescribed medicine. For this purpose, if the medicine itself does not give a distinctive test in urine, a little salicylate of sodium may be dispensed along with it. The principal drugs which we shall briefly describe are as follows:

(a) *Antipyrin*. The urine after use of this medicine may appear dicroic, being red by transmitted, and green by reflected, light. Ferric chloride, when added, produces a brownish-red colour.

(b) *Phenacetin*. In this case the urine yields a somewhat similar colour with ferric chloride. The excretion itself may be of a dark yellow tint.

(c) *Salicylic acid and its salts*. On adding solution of ferric chloride, a precipitate of the phosphates takes place, and the urine assumes a distinct violet-red colour. The colour is not unlike that given by diacetic acid, but can be distinguished from it by being demonstrable even though the urine be boiled first.

(d) *Carbolic acid, salol, tar, and creasote*. The urine in

¹ Bouchard, *Leçons sur les Auto-intoxications*, Eng. trans., by Oliver, 1895.

such cases is often dark, owing to the presence of hydro-chinon. The addition of ferric chloride yields a violet colour. If it be desired, the urine may be acidulated with 5 per cent. sulphuric acid, and distilled; the distillate gives a green colour with the iron salt.

(e) *Tannic acid*. When present in the urine the drug may be recognized by striking a greenish-blue colour with perchloride of iron.

(f) *Bromides*. These may be readily detected by setting the bromine free. To do this add to 5 c.cm. of the urine, a few drops of solution of chlorinated lime and hydrochloric acid, and a little chloroform. The latter taking up the liberated bromine, becomes yellowish-brown and sinks to the bottom of the test-tube.

(g) *Iodides*. We may demonstrate the presence of these salts in two ways: (1) Boil 5 c.cm. of urine with a particle of starch, and, when cool, run a little fuming nitric acid down the inside of the tube to liberate the iodine; a blue ring of iodide of starch will form at the junction of the two fluids. (2) Add to 5 c.cm. of urine a few drops of strong nitric acid and a little chloroform, and shake gently. The chloroform will sink and will appear of a red or violet tint from the iodine dissolved in it.

(h) *Rhubarb, senna, and chrysophanic acid*. These medicines may make the urine appear of a yellow-orange hue; the addition of an alkali, *e.g.* caustic soda, makes it distinctly red, while the red tint is once more removed by adding an acid.

(k) *Santonin*. This also colours the urine yellow, becoming red on the addition of a caustic alkali such as soda. It may be distinguished from the red colour produced in

a similar way with rhubarb, by the fact that in the latter case the subsequent addition of a reducing agent, such as granulated zinc, causes no change in the red colour, while in the case of santonin it quickly disappears.

(l) *Haematoxylin*. After the use of preparations of log-wood, the urine on becoming alkaline may show a pink tint. If an acid urine does not show this, the addition of a little ammonia may bring it out.

(m) *Vegetable alkaloids*. The examination of the urine for these substances, *e.g.* strychnine and morphine, involves processes too lengthy and complicated for clinical work. It may be mentioned, however, that several (quinine, for example) give a precipitate with picric acid soluble on heating, and one with acetic acid and potassio-mercuric iodide, soluble in alcohol. After long-continued use of morphine the urine may acquire the power of reducing Fehling's solution. In some cases this appears to be due to the presence of glucose, but in others it depends on glycuronic acid, in conjunction with which part of the morphine is probably excreted.

(n) *Copaiba*. This drug, when excreted in urine, yields, on the addition of hydrochloric acid, a reddish-purple colour, becoming more of a violet shade when heat is applied. As mentioned on p. 65, under the test for albumin, the addition of nitric acid can produce a precipitate with copaiba, but it is soluble in alcohol. Such urine is also said to be able to reduce Fehling's solution.

CHAPTER VIII.

*The urine concluded—Analysis of urinary sediments and calculi—
Unorganised sediments — Organised sediments — Calculi —
Preparation of artificial pathological urines—Bibliography.*

SEDIMENTS in urine may be conveniently studied under two heads: (1) unorganised, and (2) organised; the first class including the various crystalline and amorphous forms of salts, etc.; the second, such elements as blood-corpuscles, tube-casts, and pus-cells.

In considering these, attention must be directed both to the microscopical appearances and the chemical characters and reactions which these bodies exhibit. The general character of the urine in such cases has already been described (p. 12), but we shall now discuss the deposits themselves more minutely.

I. **Unorganised sediments.** (1) *Uric acid.* The deposit in this case is one of reddish or mahogany-coloured crystals, never very copious, but sufficiently so to be recognizable by the naked eye. The crystals are often termed “cayenne-pepper” granules, and owe their colour to the urinary pigments; in children, however, the deposit may be very pale.

Microscopically, the crystals are chiefly in the form of four-sided rhombs, whetstones, or barrels; they occasion-

ally assume the appearance of dumb-bells, or may be arranged in rosettes (fig. 5, p. 35). The urine is always acid. Chemically, they exhibit the murexide test (p. 35), and readily dissolve when the urine is made alkaline with caustic soda. They are unaffected by acetic acid.

(2) *Urates*. The amorphous deposit of quadriurates is usually of a reddish-yellow, or pink colour, the so-called



FIG. 11.—URATE OF SODIUM CRYSTALS. (Roberts.)

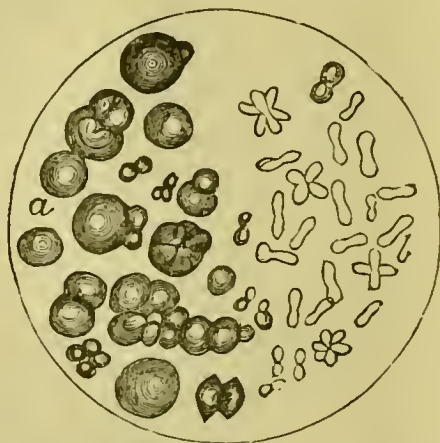


FIG. 12.—URATE OF AMMONIUM. *a*, Spheres and globular masses; *b*, dumb-bells, crosses, and rosettes. (Roberts.)

‘brick-dust’ deposit. Although, as a rule, amorphous, crystals of acid urate of sodium and ammonium may occur too, the former chiefly in children, the urine in such cases is acid, but urate of ammonium is found in alkaline urine. A rarer deposit is that of urate of calcium or magnesium.

Microscopically, the ordinary uratic deposit appears as small irregular, formless granules; if this deposit be washed, dried, and treated with water, it will be seen to dissolve, with the formation of rhombic uric acid crystals. Urate of sodium appears as balls, with many projecting spiny points, ‘hedge-hog crystals’; while urate of

ammonium has the form of spheres or balls, sometimes with projecting spikes, sometimes without (figs. 11 and 12).

Chemically, urates clear up on the application of gentle heat; they dissolve readily in alkalies; they exhibit the murexide reaction; and lastly, if treated with acetic acid, they are replaced by crystals of uric acid.

(3) *Oxalates*. The deposit of these is never very abundant, and does not form a closely cohering sediment at the bottom of the test-glass, as they are very light and sink slowly. If mucus be present the crystals may be entangled in it, conferring on it, by reason of their brightness, a somewhat glistening appearance. The urine in which they occur is nearly always acid, at least when passed, and the chemical form in which they present themselves is the calcium salt ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$). The deposit is much increased after a diet containing tomatoes or rhubarb, but all the oxalic acid present is not necessarily precipitated.

Microscopically, they appear chiefly in the form of octahedral crystals, showing two regular four-sided pyramids placed base to base. Looked at in some ways they resemble a square envelope. More rarely they are like discs which may be constricted across the short axis so as to resemble short dumb-bells (fig. 13). The crystals are often very small. Chemically, they are unaffected by acetic acid or by weak alkalies, but dissolve in hydrochloric acid.

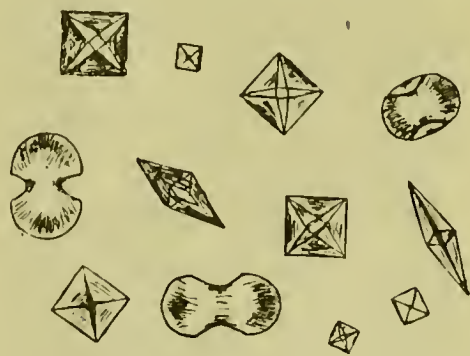


FIG. 13.—CRYSTALS OF CALCIUM OXALATE.

(4) *Phosphates*. Phosphatic deposits in general have much the same appearance, viz. that of a white flocculent precipitate very obvious to the eye, and somewhat like that of pus, which is, however, usually less mobile. The deposit may be tinged red in cases where haematuria co-exists.

Phosphoric acid is deposited in various combinations,

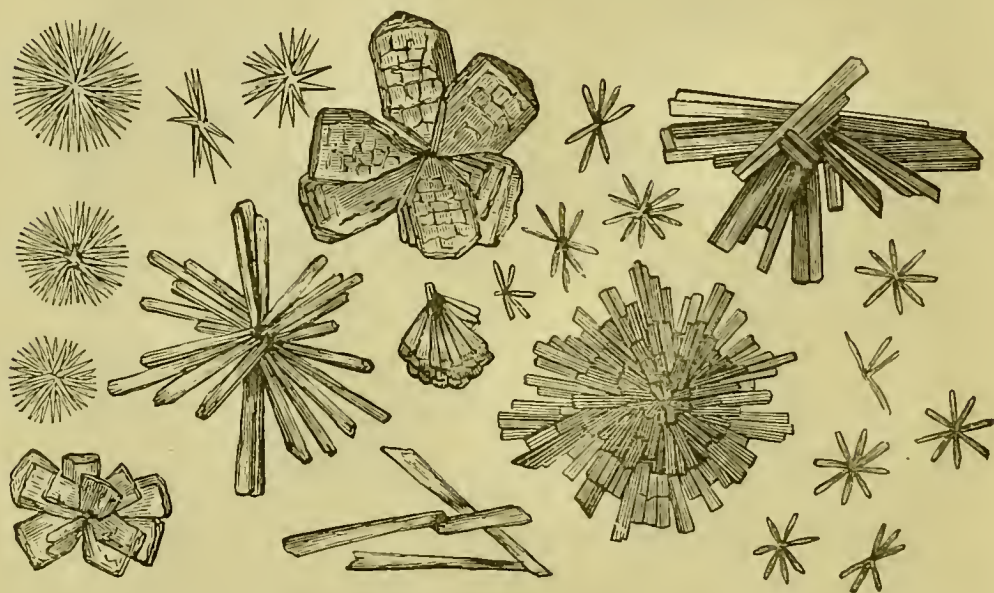


FIG. 14.—CRYSTALS OF CALCIUM PHOSPHATE. Various forms. (Finlayson.)

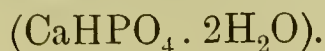
each of which has its own characters and must be considered separately.

(a) Amorphous tri-calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), mixed, it may be, with the corresponding magnesium salt ($\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$). The urine here is alkaline when passed, not from ammoniacal decomposition outside the body.

Microscopically, these salts appear merely as granular bodies. Chemically, they are insoluble in alkalis, but readily dissolved by weak mineral acids or acetic acid.

This is the deposit that sometimes appears when faintly acid urine is tested for albumin by boiling.

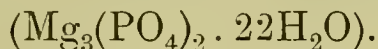
(b) Crystalline neutral calcium phosphate



This salt forms one of the rarer deposits, and is usually found in alkaline urine, but may occasionally be met with in a feebly acid urine on its way to become alkaline.

Microscopically, the crystals are in the form of rods, with square or somewhat rounded ends arranged in radiating bundles, from which they receive the name of 'stellar phosphates'; they may also assume the form of prisms (fig. 14). Chemically, they respond to the same tests as the amorphous phosphates.

(c) Crystalline tri-magnesium phosphate



This is a still rarer deposit found in weakly acid, alkaline or neutral urine.

Microscopically, it is in the form of large rhombic plates, which are bright and highly refractile. Chemically, they respond to the same tests as the two previous salts.

(d) Ammonio-magnesium phosphate ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$).

This may form a tolerably abundant white sediment, and is typically found in urines that have undergone ammoniacal decomposition. The ammonia developed in such cases unites with the magnesium phosphate, producing the double salt. The sediment is largely crystalline, and the crystals may be noticed on the surface of the urine and the sides of the test-glass. There may be a certain admixture of amorphous magnesium and calcium phosphate. Rarely the sediment is found in faintly acid urine, though it may occur in that which is neutral.

Microscopically, the crystals are easily recognized; they have the form of prisms with oblique ends, and are often called 'knife-rest' or 'coffin-lid' crystals, from their resemblance to these objects (fig. 15). More rarely they

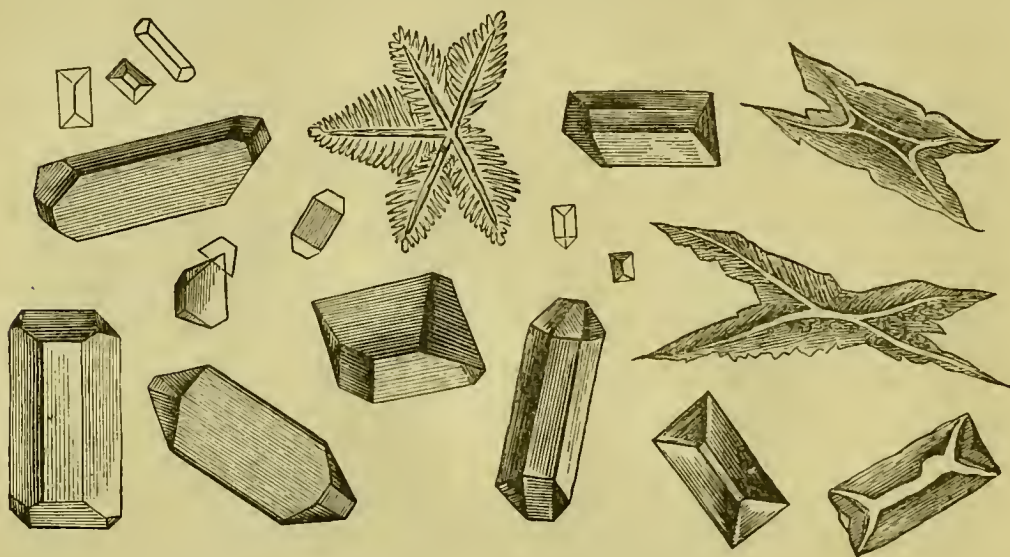


FIG. 15.—AMMONIO-MAGNESIUM PHOSPHATES. Various forms of crystals. (Finlayson.)

occur in quadri-partite feathery forms like a frost-flower. Chemically, they are readily soluble in acetic acid and in dilute mineral acids, and are unaffected by ammonia or caustic soda.

(5) *Calcium sulphate* ($\text{CaSO}_4 + 2\text{H}_2\text{O}$). This is one of the rarer urinary sediments, and has no special significance.

Microscopically, this salt forms long needle-shaped crystals with square ends, and sometimes elongated plates. Chemically, the deposit is insoluble in ammonia and acetic acid, and practically so in dilute mineral acids.

(6) *Calcium carbonate* (CaCO_3). This is also a rare deposit; it is seldom found except in alkaline urine, and may occur in that passed by persons who are vegetarians.

Graminivorous animals, such as the horse and ox, deposit it readily in their urine.

Microscopically, it does not form true crystals, but rather granular concretions and small dumb-bell shaped masses. Chemically, it is unaffected by alkalies or ammonia, but dissolves readily in acetic acid and mineral acids with effervescence due to carbonic acid gas.

(7) *Hippuric acid*. This is not a common deposit, but may be observed in the urine after doses of ammonium benzoate, or a diet of cranberries.

Microscopically, the crystals are in the form of somewhat long four-sided prisms. Chemically, they are insoluble in acetic and in hydrochloric acid; soluble in ammonia.

(8) *Leucin and tyrosin*. The chemical and microscopical characters of these two bodies have already been described (p. 108).

The student is reminded of the fact that they do not as a rule occur as a deposit, but must first be extracted from the urine.

(9) *Cystin*. This also has been described on p. 110, where its crystalline form and chemical reactions will be found. It may exist in solution in urine from which it can be precipitated by acetic acid.

(10) *Xanthin* ($C_5H_4N_4O_2$). This is a very rare deposit which may cohere to form a calculus.

Microscopically, xanthin forms elongated oval crystals like whetstones. Chemically, it is soluble in ammonia, insoluble in acetic acid.

(11) *Cholesterin* ($C_{27}H_{45}OH$). This is an extremely rare constituent of urine, though it is abundant in bile. It has been found deposited from urine.

Microscopically, it occurs in large flat tabular crystals, which often look as if a square corner had been snipped

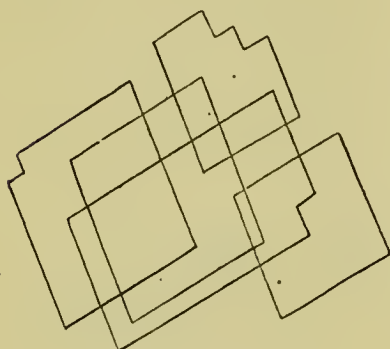


FIG. 16.—CHOLESTERIN CRYSTALS.

out (fig. 16). Chemically, if heated with strong sulphuric acid it is gradually eaten away with the formation of a crimson colour, while with iodine and sulphuric acid it gives a play of colours — red, violet, and green.

(12) *Indigo-blue*. This sometimes occurs in concretions, sometimes as crystals, in urine undergoing alkaline decomposition. The crystals are coloured blue. Microscopically, they are usually in the form of fine radiating groups of needles.

II. Organised sediments. These include mucus, tube-casts, red blood cells, leucocytes, pus and epithelial cells, fat globules, microbes and low organisms, spermatozoa, and fragments of morbid growths. In their detection and differentiation the microscope is more to be relied on than any chemical tests.

(1) *Mucus*. This presents no organised structure under the microscope, but may have entangled in it epithelial cells and leucocytes, or crystals of calcium oxalate.

It sometimes assumes the form of elongated or ribbon-like coagula, which look like narrow flattened tube-casts; occasionally pus-cells glued together by a mucinoid material give rise to the idea that they are threads of organised structure. These latter are sometimes called 'gonorrhœa threads.' If a little dilute acetic acid be

added, the mucus and the nuclei of the pus-cells become more distinct.

(2) *Tube-casts*. These are among the most important of the organised urinary deposits, and sometimes, though not often, form a sediment apparent to the naked eye. They are, as a rule, indicative of morbid changes in the

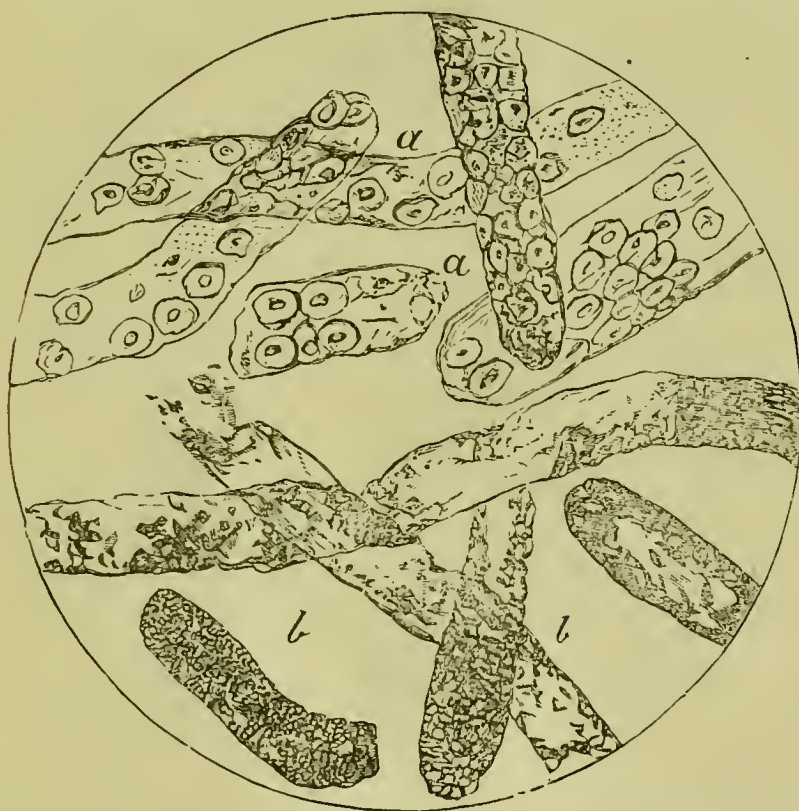


FIG. 17.--TUBE-CASTS. *a*, Epithelial; *b*, granular. (Roberts.)

kidney, and are found most commonly of all in acute nephritis; but it must be kept in mind that tube-casts may occur in urine free from every trace of albumin, and in persons apparently in perfect health.

The casts are formed, as their name suggests, within the urinary tubules, which act the part of moulds. There are several varieties of casts, which may be considered in brief detail:

(a) *Epithelial*. These are built up of cells shed from the urinary tubules and held together by some cement or ground-substance; the cells are flat and polygonal, the nuclei generally distinct (fig. 17).

(b) *Blood-casts*. These result from the effusion of blood into the renal tubules, and may be composed of red cells



FIG. 18.—TUBE-CASTS. *a*, Fatty casts; *b*, *c*, blood casts; *d*, free fatty molecules. (Roberts.)

or leucocytes, or both massed together, to form a cast (fig. 18).

(c) *Granular*. These are the result of cellular disintegration within the tubules; their character is sufficiently indicated by their name (fig. 17).

(d) *Waxy*. These are somewhat translucent or clear, and fairly large and broad. They are composed of material that has undergone amyloid degeneration.

(e) *Hyaline*. The origin of this variety of casts is still *sub judice*; they also are clear, but not so large as the waxy casts, though they are somewhat broader.

Some writers also describe fatty casts and a still rarer variety composed of bacteria.

(3) *Red blood-corpuscles*. The sediment formed by these is reddish-brown, but so little blood may be present that none is deposited unless the fluid is centrifugalized; in such cases the urine usually looks smoky. Microscopically, the corpuscles may be abundant or scanty, normal, swollen, or shrivelled, red in colour or of the palest yellow hue. Leucocytes may be mixed with the coloured cells.

(4) *Epithelial cells* (fig. 19). Very commonly in urine some flat squamous cells are found which take origin in the

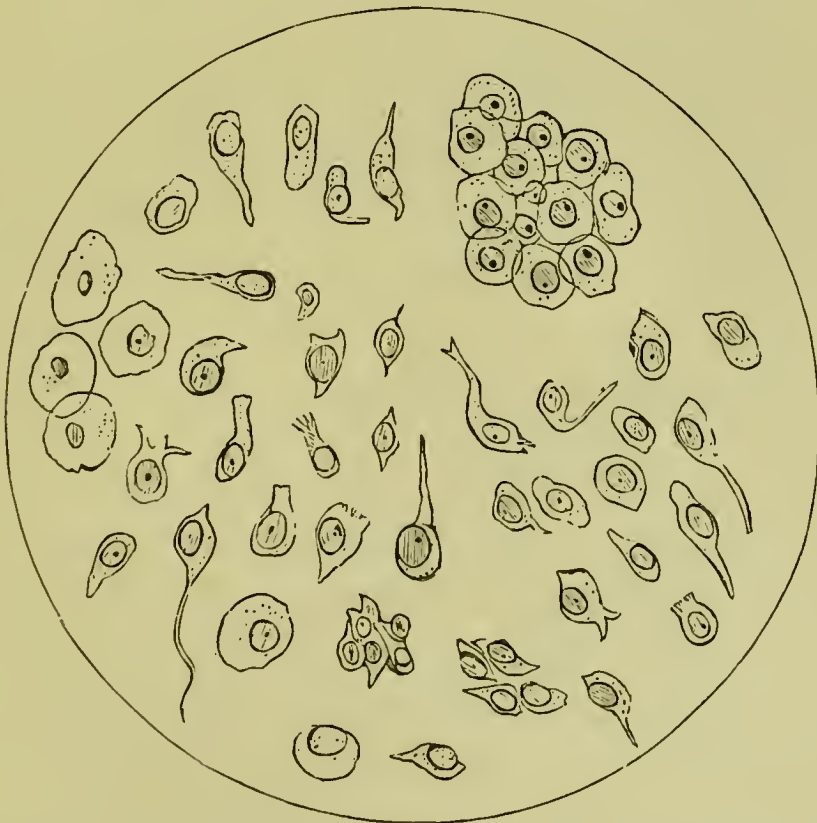


FIG. 19.—EPITHELIAL CELLS FROM THE BLADDER, URETER, AND PELVIS OF KIDNEY. (Roberts.)

urethra near its meatus, or in the vagina in females, and which are coincident with perfect health. Again, we may find cells that are columnar or cylindrical, and somewhat tailed, and which form the surface lining of the urethra in most of its extent, while those situated on the mucous lining of the bladder, ureter, and renal pelvis are rather more conical. On the very surface of the mucosa may be found a layer of polygonal or rounded cells. Finally, cells from the renal tubules themselves are polyhedral, granular, and provided with a large nucleus.

(5) *Pus*. This may form an abundant sediment of a white, whitish-yellow, or whitish-green colour, much less mobile than the deposit of phosphates. It is often of a slimy consistency, and is the more so if the urine be alkaline.

Pus occurs in gonorrhœa, cystitis, pyelitis, and from rupture of an abscess into some part of the urinary tract. It may be found also as the result of accidental admixture of the vaginal secretion in women. It is generally stated that when the urine is alkaline, the *pus* probably comes from the bladder, and when acid, from the pelvis of the kidney.

Microscopically, *pus* cells are easily recognized; by treatment with a little dilute acetic acid the nuclei are rendered more distinct. They may resemble round epithelial cells, and v. Jaksch recommends the addition of a little iodine dissolved in iodide of potassium to distinguish between the two. This reagent stains *pus*-corpuscles a deep brown (glycogenic reaction), while epithelial cells only become yellow. Chemically, Donne's or Vitali's test may be employed. In the first the urine is decanted off from the sediment, which is in its turn poured into a test-tube

so as to half fill it. On the addition of strong solution of caustic potash the purulent mass is changed into a slimy, gelatinous, 'ropy' substance.

Vitali's test is performed by filtering some of the urine (acidulated with acetic acid if it be alkaline), so that the pus is retained on the filter-paper. Over it is poured a thin layer of fresh active tincture of guaiac, when a blue coloration will become apparent.

(6) *Fat-cells*. These are not commonly met with in urine, though they are found occasionally, notably in the condition called chyluria, where they may make the urine turbid. The special name for the condition where fat exists in urine is 'lipuria.' Microscopically, they appear as globular bodies, endowed with a high degree of refractility. Chemically, they are soluble in ether.

(7) *Microbes and other low organisms*. Various micro-organisms are found in urine. In that which is undergoing ammoniacal decomposition, large numbers of bacteria may be present, as well as the micrococcus ureæ, arranged in groups or chains. In diabetic urine which is fermenting, yeast-cells may be found, and they may be replaced later by moulds. Sarcinæ also occur in urine, smaller than those found in the stomach. In addition to these more harmless germs, pathogenic microbes may occur, notably the tubercle bacillus of the urinary tract, and the gonococcus (see bacteriuria, p. 113). Of other low forms of life, the most interesting and important, perhaps, is the distoma hæmatobium, which causes hæmaturia in tropical countries, and whose oval ova, mixed with blood-corpuscles, may be detected in the urine.

(8) *Spermatozoa*. These may be found in the urine

under various conditions, and can be readily enough recognized by the oval head and slender tail-like extremity.

(9) *Fragments of pathological tissue.* It is frequently stated that useful aid in diagnosis may be obtained from the finding of cancer cells or particles of villous tumours in the urine. This, however, appears to be a somewhat rare occurrence, and much caution should be exercised in drawing any conclusion from it.

(10) Lastly, foreign bodies may frequently be found in urine, and are often misleading. The principal objects that occur are hairs, fibres of cotton and linen, wool, and amorphous particles of dust and carbon. The urine may be contaminated by the patient expectorating or vomiting into the vessel containing it, and in cases where the catheter has been used, oil globules are not uncommonly present.

Preservation of urinary sediments. It is often desirable to preserve organised urinary sediments, and this can be done by the method advocated by Fischel.¹ In this the sediment is first washed two or three times with normal saline solution (0.75 per cent.), best with the aid of the centrifuge, and then preserved in a mixture of equal parts of glycerine and water, containing 2 per cent. of a saturated alcoholic solution of thymol. To examine, a drop of the mixture is placed on a slide, covered, and surrounded with a ring of varnish or vaseline to prevent evaporation.

Analysis of urinary calculi. The chief varieties of calculi are (1) uratic, (2) phosphatic, (3) oxalic; (4) in addition to these, mixed calculi may occur, *e.g.* those with a uric acid nucleus and a phosphatic accretion; and (5) those composed of cystin, xanthin, etc.

¹ *Amer. Jour. of Med. Science*, p. 249, vol. cxi., 1896.

(1) *Uratie calculi* may be composed of urates, or of uric acid, or of both. In consistency they are hard, and they are of the colour of the uratic deposit, yellow or reddish-yellow. For analysis a small one should be broken up.

Tests. (a) Heated on glowing platinum foil they are oxidized away, leaving little residue.

(b) Heated gently with dilute hydrochloric acid they do not dissolve.

(c) They give the murexide test.

(2) *Phosphatic calculi.* These are composed of earthy phosphates and of ammonio-magnesium phosphates. They are white or grey in colour, with a rough exterior, and are softer than the uratic.

Tests. (a) They burn a little when heated on platinum foil, owing to combustion of the organic ground-substance of the calculus, but most of the fragment is left, in a blackened condition, insoluble in water.

(b) Heated with a little dilute hydrochloric acid, they are dissolved; with acetic acid they exhibit the same behaviour. In both cases ammonia in excess causes a re-precipitation of the earthy phosphates.

(3) *Oxalic acid.* This, as oxalate of calcium, forms a very hard calculus, often nodulated or mulberry-like, and of a light-brown colour, which may be darker if altered blood be incorporated with the stone.

Tests. (a) Heated on platinum foil it also leaves a black residue, consisting now of calcium carbonate from combustion of the organic salt. (A calculus of calcium carbonate will behave similarly on being heated.)

(b) A fragment warmed with dilute hydrochloric acid will dissolve; the residue left after combustion, treated

in the same way, will dissolve with effervescence due to escape of carbonic acid gas. In the former case the addition of ammonia in excess will cause the oxalate to re-precipitate, but its addition to the dissolved residue will cause no precipitate.

(4) *Mixed calculi* often exhibit a lamellar or stratified structure when sawn across; they respond to the tests of two or more of the simpler substances.

(5) *Cystin calculi* are yellow and very hard in texture.

(a) Heated on platinum foil alone, they burn with a greenish-blue flame.

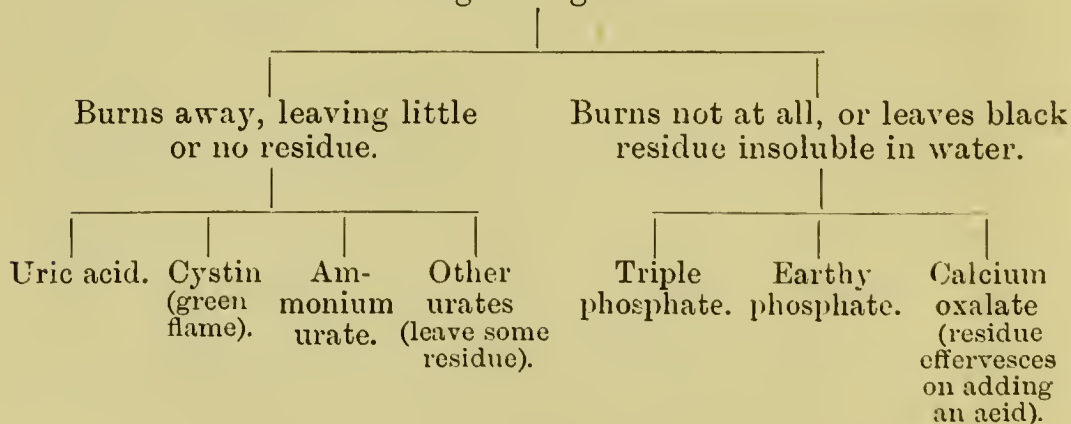
(b) Heated on the foil with a little caustic potash and plumbic acetate, they leave a black mark, due to sulphide of lead.

(c) When warmed with dilute hydrochloric acid, they dissolve; the addition of ammonia causes no precipitate. They are unaffected by acetic acid.

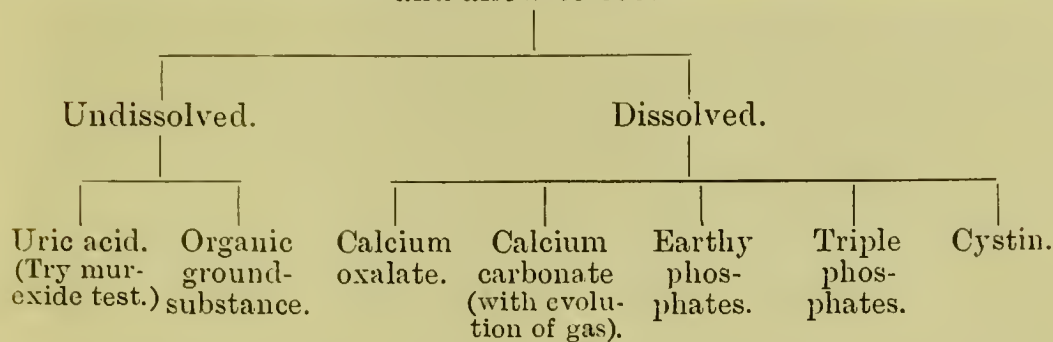
Analysis of a calculus of unknown composition (arranged from Tappeiner).

Reduce the calculus to powder; if large, saw it into pieces of suitable size.

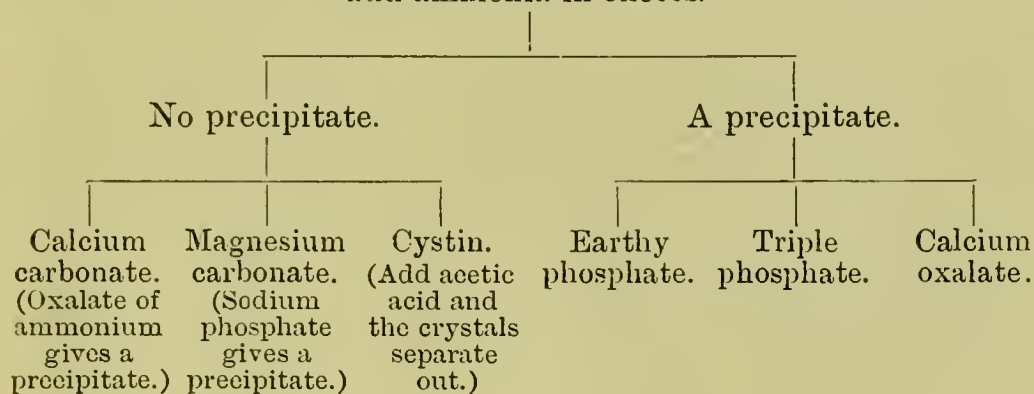
I. Heat some of the powder on platinum foil till the latter begins to glow.



II. Heat a second sample with dilute hydrochloric acid gently and allow to cool.



III. In order to differentiate the substances which went into solution above, filter the solution, dilute well with water, and add ammonia in excess.



IV. If treated with acetic acid till faintly acid, the earthy and ammonio-magnesium phosphates dissolve, while calcium oxalate remains undissolved. Cystin separates out slowly in six-sided plates.

Preparation of artificial pathological urines.—It is often desirable, in the absence of genuine specimens, to prepare artificial urines for the purpose of examinations, demonstrations, etc. It may therefore be advantageous to give a short account of the best ways of preparing imitations of the chief pathological urines.

In the first place, let it be noted that the addition of a few drops of chloroform to a urine will preserve it for a considerable time. The urine should be placed in a flask, well shaken with the chloroform, and then corked up.

(1) *Albumin.* To prepare albuminous urine, take the white of a hen's egg, clip it thoroughly with scissors to divide the fibrous septa and shake up thoroughly in a flask with 100 c.cm. (about 4 oz.) of water. After allowing any solid particles to settle, take 20 drops of the supernatant fluid, add this to 100 c.cm. of urine and shake. Such a urine will answer well to the various tests for albumin. (Both albumin and globulin are present here.)

(2) *Sugar.* This is easily simulated by dissolving 2 grms. of grape-sugar in water by the aid of gentle heat, and then shaking up the solution with 100 c.cm. of urine.

(3) *Blood.* This may be added directly to the urine, which it will, of course, colour. Artificial phosphates may be added to imitate a urine with phosphatic deposit coloured red by hæmoglobin.

(4) *Phosphates.* To prepare a urine rich in phosphates, and which will deposit them on boiling, proceed as follows: Mix together some solution of carbonate of sodium and chloride of calcium, when a precipitate of calcium carbonate will quickly form and sink to the bottom of the glass; pour off the clear fluid, wash the precipitate with water, and again decant this off. Now add a sufficiency of the precipitate to the urine and shake well; on filtering, a clear urine will be obtained so rich in phosphates that it will deposit them when boiled, and more quickly still if ammonia be added, when a precipitate comes down simulating that in ammoniacal urine.

(5) *Bile.* It is not easy to prepare a good artificial bilious urine. One may obtain some fresh ox-bile and shake up a little very thoroughly with urine. The latter

will then resemble that from a case of choluria, in naked-eye appearance, but does not give Gmelin's test satisfactorily. It does, however, show a green ring with tincture of iodine.

(6) *Urobilin*. To procure this substance, macerate a little fresh fæces in a small beaker with, say, 10 c.cm. of absolute alcohol, and allow to stand covered for an hour. On filtering, a brown-red solution of urobilin will be obtained, and may be added to urine, where, with zinc chloride and ammonia, it will show the characteristic green fluorescence.

(7) *Uric Acid*. The free acid may be precipitated from some urine by strongly acidulating with pure hydrochloric acid, and allowing to stand for a day. Such a urine often looks very dark, so some of it may be decanted off, filtered through animal charcoal, and returned to the rest.

(8) *Urates*. These, as they occur in the ordinary brick-dust deposit, are not easily prepared artificially; if to urine we add enough ammonium chloride, a precipitate of yellow-red ammonium biurates quickly forms, but this, though it looks like the ordinary amorphous deposit of quadriurates, differs from it in not being dissolved on heating. A true deposit of quadriurates can be obtained after Roberts' method, as follows: Dissolve 3 grms. of potassium acetate in 100 c.cm. of urine, heat in a flask to the boiling point, and shake up with excess of pure uric acid for a minute or so. Filter the mixture quickly and cool the filtrate under a running tap of cold water, when a bulky precipitate of amorphous quadriurates forms.

(9) *Acetone and di-acetic acid.* Acetone may be added direct to the urine, say 20 drops to 50 c.cm., and used for Gunning's or Lieben's test.

Aceto-acetic ether may also be added to urine to show the perchloride of iron test.

(10) *Indican.* Lassar-Cohn recommends an alcoholic extract of indican to be made from horse's urine, which, he says, will keep for years. A quantity of the urine is evaporated to dryness on a water-bath, and the residue extracted with alcohol, which removes the indican. This extract is allowed to stand for 24 hours and then filtered, whereby a clear alcoholic solution of indican is obtained, of which a little may be added to the urine.

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¹ No attempt is made here to give a complete bibliography; the author has merely indicated a few standard works of reference, of recent publication, which the student may find useful.

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SECTION II.

ANALYSIS OF GASTRIC FLUIDS.

CHAPTER I.

Method of obtaining specimens—Use of stomach tube—Test-meals—Examination of stomach-contents—Naked-eye appearances—Odour—Quantity—Microscopical appearances—Reaction—Total acidity—Hydrochloric acid—Qualitative and quantitative tests—Acid salts.

THE use of chemical methods in the diagnosis of gastric disorders is a comparatively recent development, at least in this country, but their importance and value are quickly becoming recognized, and their employment is now very common. It is, indeed, as rational that we should seek, by chemical examination of the stomach contents for information to aid us in the diagnosis of gastric disorders as that we should appeal to urinary analysis in the case of affections of the kidneys.

At the outset, however, we are confronted with a difficulty. No trouble is experienced in getting specimens of the renal secretion several times a day if desired, and under varying conditions; but, save where vomiting

occurs, we must have recourse to artificial aids in order to obtain samples of the stomach contents. The means which are employed consist of the soft stomach-tube, by the help of which we can evacuate the stomach at any time desirable. The tube, which is about a metre and a half long and 1.5 cm. in diameter, is made of red rubber, and may terminate either in a rounded solid end with an eye or opening 2 cm. up the tube, or may end as if cut straight across. In the latter case there is a little risk of the gastric mucosa being sucked against the opening, and so closing it. There is usually a mark on the tube 45 cm. (18 in.) from its extremity indicating the average distance from the teeth to the cardiac orifice of the stomach. In passing the tube, it should be passed directly backwards in the middle line against the posterior pharyngeal wall, the patient meantime sitting, with the mouth open and the head slightly protruded forward. Any spasm or resistance is best overcome by asking the patient to take a deep breath. Once the tube reaches the gullet proper the patient should be asked to perform the movement of swallowing, when the tube will usually easily find its way into the stomach. Samples for examination may be obtained by attaching the tube to the moderately exhausted bottle of an aspirator, or the tube may have a bulb-like expansion on it whereby the fluid may be pumped up from the stomach.

It is obvious that the meal last ingested will greatly influence the composition and condition of the sample thus obtained, and it has therefore been found desirable to employ what is termed a 'test-meal.' By this means we know what and how much we intro-

duce into the stomach, and what we may expect to evacuate.

Of these test-meals there are several types:

(1) Ewald's consists of 35 grms. ($1\frac{1}{3}$ oz.) of bread, and one-third of a litre of water. The stomach is emptied one hour thereafter.

(2) Leube advocates a dinner of a little soup, a large beef-steak (say 5 oz.), and a dinner-roll; five hours after this the healthy stomach contains an acid fluid in which peptones are present, and seven hours after it a little neutral fluid and no food, peptones, or acid.

(3) Germain Sée recommends 60 grms. ($2\frac{1}{2}$ oz.) of meat, 100 grms. (4 oz.) of bread, and a glass of water—the stomach to be emptied in two and a half hours, when digestion should be at its height.

The ideal conditions under which a test-meal is administered are, that we should first wash out the stomach by the syphon-tube, next administer the meal, and, finally, evacuate the organ at the desired time. Most patients, however, object so strongly to this procedure that the preliminary lavage must be abandoned as a rule.

The fasting stomach usually contains a little neutral fluid (or it may be alkaline from swallowed saliva) in which a few flakes of mucus may be suspended. Schule,¹ however, in 31 out of 34 trials on the fasting stomach, obtained a few cubic centimetres of an *acid* fluid, and occasionally peptones and pepsin.

During digestion there is a certain amount of absorption of peptones, alcohol, salts, etc., from the stomach, but the great bulk of the chyme is handed on to the

¹ *Berl. klin. Wochens.*, No. 52, 1895.

intestine to undergo further chemical changes, and finally to be absorbed therefrom.

Accordingly, when food is found in the stomach after the period at which the organ should be empty, it is usually due to deficient peristalsis and want of motor power, and this is found typically in dilatation, with or without bacterial fermentation, in pyloric obstruction, and to a lesser degree in chronic catarrh.

It may happen, however, that we must rely, not on material removed by the stomach-tube, but on that which is vomited. In this case we must carefully inquire into the nature of the meal last ingested, and the time thereafter that the stomach rejected its contents. For the purpose of chemical analysis, the matter vomited 2-2½ hours after food is very suitable, and the same holds good if the tube be used after a test-meal of bread, meat, and water.

I. Physical examination of gastric contents. (1) *Appearance to the naked eye.* The fluid may, for this purpose, be conveniently placed in a urine-glass, and it will be noticed that the solid particles, on account of their size, tend to subside quickly, unless much mucus, easily recognized by its glairiness, be present entangling them. The colour is normally whitish-yellow, but it may be green or greenish-white from the presence of bile, bright red or plum-coloured from freshly-effused blood, or dark brown, even black, from altered blood, constituting then what is known as 'coffee-grounds' material. The fluid may be copious and light brown in colour, with a scum floating on the top, in cases of bacterial fermentation, and pus is occasionally found in cases of phlegmonous gastritis. The size and

general appearance of the solid particles, the presence or absence of curdled milk, of foreign bodies, undigested food, and so on, should be carefully noted.

(2) *Odour*. Normally the fluid possesses what may be termed a "gastric" odour. In cases of dilatation with fermentation the smell may be yeasty or beery. Where intestinal obstruction has occurred it may present a very disagreeable and fæculent odour. Drugs administered to the patient, or poisons taken by him, may impart their peculiar smell, *e.g.* carbolic acid, prussic acid, turpentine, and chloroform, and the presence of alcohol may often be detected in a similar way.

(3) *Quantity*. Attention should also be paid to this point. The amount rejected varies greatly, but is often very large in cases of pyloric obstruction and dilatation, when many pints of fluid may be vomited or drawn off by the tube.

(4) *Microscopical appearances*. A little of the sediment should be removed by a pipette and examined microscopically. The objects found may comprise starch granules, muscle fibres, fat globules, and fatty crystals, blood-corpuscles, yeast cells, sarcinæ, bacteria, pus cells, and fragments of pathological tissue (fig. 20). Naturally, the detection of one or other of these structures may throw much light on the nature of the disease the patient suffers from, sarcinæ and yeast cells, in particular, being associated with fermentation of the stagnant contents of an enlarged atonic organ. A little of the fluid may be smeared on a cover-glass to form a film, and when dry, stained, as Sidney Martin recommends, in a very dilute watery solution of gentian violet, which often brings out the sarcinæ

very distinctly. These little organisms, whose full name is *sarcinæ ventriculi*, occur very commonly in cases of

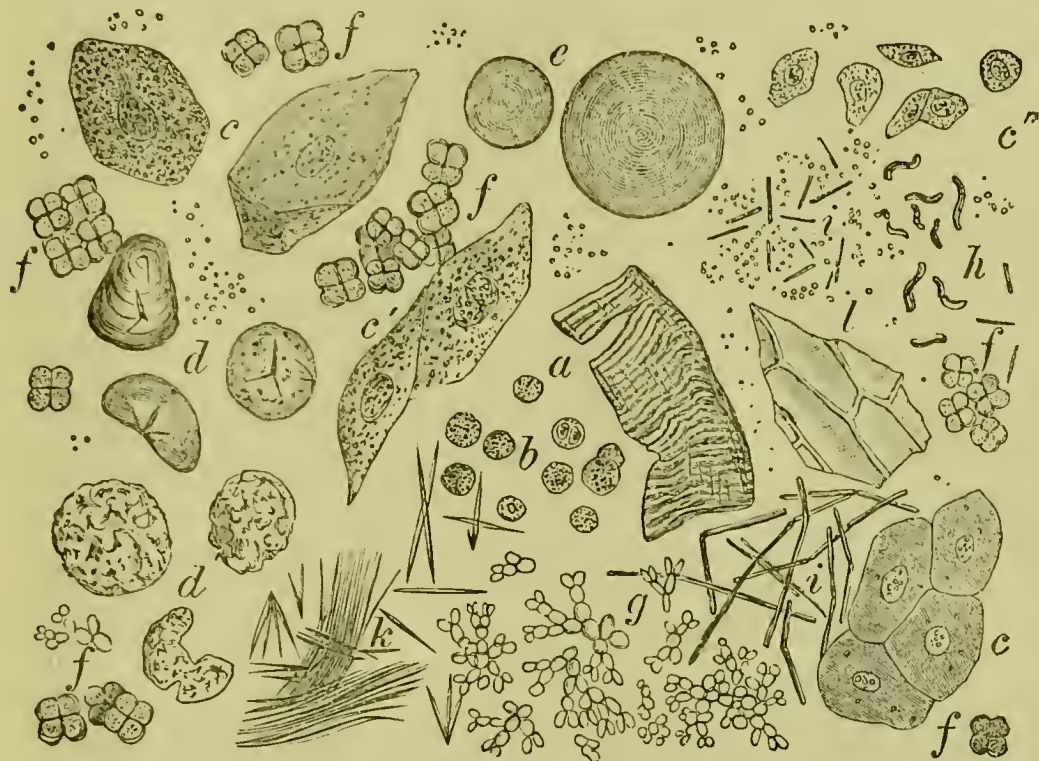


FIG. 20.—MICROSCOPICAL VIEW OF VOMITED MATTER. *a*, Muscle fibres; *b*, white blood-corpuscles; *c*, squamous epithelium; *c'*, columnar epithelium; *d*, starch grains; *e*, fat-globules; *f*, *sarcinæ ventriculi*; *g*, yeast fungi; *h* and *i*, bacilli and micrococci; *k*, fat-crystals and connective tissue; *l*, vegetable cells. (v. Jaksch.)

dilated stomach, and under the microscope present a characteristic appearance. They occur arranged in groups of four or eight, bearing a close resemblance to bales of wool (fig. 20 *f*). They seem to secrete some acid themselves, but are not the cause of the high acidity of the gastric contents in cases of gastriectasis.

II. Chemical examination. Healthy gastric juice contains 99·4 per cent. of water and 0·6 per cent. of other elements, of which there is about 0·33 per cent. of organic matter (chiefly pepsin), 0·02-0·2 per cent. of hydrochloric acid, and 0·1 per cent. of sodium chloride, the balance being composed chiefly of other chlorides and of phos-

phates. The specific gravity is 1.002-1.006. The essential constituents, as far as digestion is concerned, are hydrochloric acid, pepsin, and the rennet or milk-curdling ferment.

In order that correct conclusions may be drawn from gastric analysis, the student must first understand clearly what takes place in a healthy stomach after ingestion of a meal, say of bread, meat, and water.

The digestive processes, as regards the presence of acids, may be divided into three stages, pepsin being present throughout. The following scheme is given by Lockhart Gillespie :

First Stage.—Free HCl absent; combined HCl present; lactic acid present or absent; ptyalin still acts.
Duration— $\frac{1}{2}$ - $\frac{3}{4}$ hour.

There being in this stage no free and little combined hydrochloric acid—itsself a valuable gastric antiseptic—some lactic acid may be formed by bacterial agency from maltose or starch, and ptyalin in the saliva swallowed may still act on starch. Any hydrochloric acid secreted during this stage goes into loose combination with the proteid matter present, and is termed “combined” hydrochloric acid.

Second Stage.—Free HCl now present; acidity rising; lactic acid absent or going; ptyalin no longer acts. Duration— $\frac{3}{4}$ hr.-3 hrs. after food.

During this period the proteids taken become saturated with hydrochloric acid, and some of the latter now remains free and uncombined. The total acidity is rising in intensity, but the lactic acid is being absorbed and lost. Ptyalin can no longer act.

Third Stage.—Acidity falling; more free HCl in proportion to the combined; lactic acid absent.

Duration—3-5 hours after food.

The mass of food, containing much combined hydrochloric acid, is now passing out of the organ, so that the total acidity falls, while the free acid is relatively increased. No lactic acid is found. In all three stages, albumoses, peptones, and maltose are present. We are now prepared to consider the chemical analysis in detail.

(1) *Determination of reaction of the fluid.* If blue litmus paper be dipped in the fluid it almost invariably turns red, showing that it is acid. But acid phosphates (*e.g.* NaH_2PO_4) will produce this effect, and these are nearly always present to some extent. It is therefore necessary to use some other reagent in order to find out whether free acids are present as well. Such a reagent, among many others, is Congo-red, an aniline dye. Strips of filter-paper soaked in a strong watery solution are of a bright red colour, which turns blue only with free acids, not with acid salts. Organic acids, such as acetic, tend to make the paper of a violet-blue shade; with hydrochloric acid it becomes dark-blue.

(2) *Determination of total acidity.* If the stomach contents are very fluid, they may be used as they are; if much solid matter be present, straining through muslin or cheese cloth is advisable as a preliminary.

For the purpose of estimation, a deci-normal solution of caustic soda in distilled water (4 grms. to the litre) is employed. This is, of course, the equivalent of a similar solution of hydrochloric acid (which contains 3.65 grms. in the litre), and every c.cm. of the soda solution will

therefore neutralize and be equal to 0.00365 grms. of hydrochloric acid.

To carry out the estimation, a burette is filled with the soda solution and fixed in the clamp; 5 c.cm. of the gastric fluid are now accurately measured out by a pipette and placed in a porcelain dish. The outside of the pipette having been wiped, the interior is rinsed out into the dish by a jet of water, and the whole stirred. It is necessary to have an indicator, and this is supplied by adding a few drops of a 2 per cent. solution of phenolphthalein in absolute alcohol, which, colourless in itself, turns a vivid pink with an alkali. The soda solution is now run cautiously into the dish, with constant stirring, till a permanent pink tint appears. A little time must always be given to allow the soda to neutralize the combined hydrochloric acid. The quantity of soda used is now read off and the calculation made.

Ex. Suppose 3.7 c.cm. have been run from the burette, then $0.00365 \times 3.7 = 0.0135$ grms. HCl in 5 c.cm. of gastric fluid, or $0.0135 \times 20 = 0.27$ per cent.

The result is given in terms of hydrochloric acid, but it must be borne in mind that the acidity may be due to this acid, or to organic acids such as lactic or acetic, or to acid salts, or to these bodies in any combination.

Ewald suggests another way of stating the acidity. If 5 c.cm. of the fluid were neutralized by 5 c.cm. of the soda solution, or, in other words, 100 c.cm. of the one by 100 c.cm. of the other, he would state the acidity as 100. In the example given above, where 5 c.cm. required 3.7 c.cm. of soda, 100 would require 3.7×20 or 74. The acidity in this case would be 74. If three hours after a

meal the acidity is over 0·36 per cent., we may regard the condition as one of hyperacidity. In cases of fermentation, with copious formation of organic acids, it may rise to over 0·5 per cent. The gastric juice is also hyperacid in round ulcer of the stomach, and in the condition termed Reichmann's disease (hyperchlorhydria). The acidity is diminished in cancer of the organ (unless there be retention of contents and fermentation), in chronic catarrh and atrophic conditions of the mucous membrane, in hypertrophic cirrhosis of the liver, and in febrile conditions. It is also temporarily lessened by the ingestion of large quantities of fluids, especially if these contain alkalies.

Hydrochloric acid. This, the essential acid of digestion, has tests of its own which are of importance in view of the fact that most physicians agree that in cancer of the stomach the majority of cases show no free hydrochloric acid 2-3 hours after a meal. The value of this as a diagnostic sign is admitted now by most clinicians, including Ewald, v. Jaksch, Soltau Fenwick, Hale White, Sahli, Osler, etc.

The average normal amount of hydrochloric acid is, according to Richet, about 0·18 per cent. It is increased in Reichmann's disease (true hyperchlorhydria), and in at least some cases of round ulcer of the stomach. It is diminished in cancer, chronic catarrh, acute catarrh, chronic ulceration, and in some general affections such as tuberculosis.

Tests for free hydrochloric acid. (I.) *Qualitative.* A great number of these have been introduced, but only four or five will be given here, of which the author has

had personal experience and which may be regarded as delicate and reliable.

(1) *Gunzberg's*. The reagent employed is composed of phloroglucin, 2 grms., and vanillin, 1 gm., dissolved in 30 c.cm. of absolute alcohol. It should be kept in a well-stoppered bottle and preserved from the light.

To perform the test, pour two or three drops of the reagent into a small porcelain dish, add a drop or two of the filtered stomach contents, mix and evaporate carefully to dryness over a spirit lamp, avoiding charring. If the free acid be present, as soon as the mixture is dry a delicate rose or carmine tint will appear, clearly visible against the white of the dish. If no free hydrochloric acid exist, the mixture will simply leave a whitish-yellow film.

The organic acids as they occur in gastric contents do not produce any colour change. This test is distinctive, delicate, and reliable, and can with certainty detect 1-2000 of the acid. The ingredients however, are somewhat costly.

(2) *Boas's test*. This is made by dissolving 5 grms. of resorcin along with 3 grms. of cane-sugar in 100 c.cm. of weak alcohol (45 per cent. B.P.). The test is performed as with the reagent first described, viz., by evaporation to dryness along with a little of the stomach contents. Free hydrochloric acid shows itself by a red coloration. The test is good and the reagent inexpensive.

(3) *Tropælin OO test*. This is performed by dissolving a little (say half a gm.) of this aniline product in 30 c.cm. of methylated spirits and filtering; the result is a clear, yellowish-brown solution. A few drops of this are evaporated to dryness in a porcelain dish, but at a gentle

temperature (scarcely above body-heat). A drop or two of gastric fluid added will cause the appearance of a purple tint if free hydrochloric acid be present. This test may very conveniently be applied by strips of filter paper dipped in the test solution and dried. They have then a yellow-brown colour, and when placed in a solution containing free hydrochloric acid become dark brown, turning to violet or purple when gently dried over a small flame. The test is reliable and the ingredients of the reagent are cheap.

(4) *Toepfer's test*. This is said to be the most delicate test yet introduced. The reagent consists of a 0.5 per cent. solution in alcohol of an aniline compound named di-methyl-amido-azo-benzol. To perform the test, filter a little of the gastric juice, and of the filtrate place 5-10 c.cm. in a small beaker on a white ground. On adding two drops of the test solution, the development of a distinct red or pink colour indicates the certain presence of free hydrochloric acid. It is said that as little as 1-5000 of the acid can be detected by it.

II. **Quantitative estimation of hydrochloric acid.** This cannot be done very easily for clinical purposes, though there are numerous laboratory methods. Probably the simplest reliable processes are those devised by Leo and by Toepfer, and they alone will be described here. (Hehner and Seemann's method will be described under "Organic acids," p. 157.)

(1) *Leo's method*. This is based on the fact that calcium carbonate can be neutralized by the hydrochloric acid of the gastric juice, but not by the acid salts. If, therefore, the acidity is estimated before and after the addition of

calcium carbonate, the difference will indicate the amount of hydrochloric acid present, provided always that organic acids are not present in the fluid. (If they are they must be estimated separately, see p. 157.)

To perform the estimation, take 10 c.cm. of the filtered stomach contents, add 5 c.cm. of strong solution of calcium chloride and a few drops of phenol-phthalein solution, and estimate the acidity in the usual way with $\frac{N}{10}$ caustic soda; note the result which gives the acidity due both to hydrochloric acid and to acid salts.

Now take 15 c.cm. of the filtered juice and rub up thoroughly with one gramme of dry powdered calcium carbonate; a slight escape of carbonic acid indicates the effect of the acid present on the calcium salt. The white creamy mixture must be filtered (through asbestos preferably), and of the filtrate 10 c.cm. (which now contain no free acid save carbonic dioxide) are placed in a small flask. A cork with two holes is fitted in, through one aperture of which a glass tube passes down to the bottom of the flask, while the other transmits a short bent tube connected with an aspirator. A current of air is made to bubble through the fluid, and in this way the carbonic acid is driven off.

The liquid is now treated as before with 5 c.cm. of calcium chloride solution and some phenol-phthalein and again titrated with $\frac{N}{10}$ caustic soda, and the result noted.

The acidity will be less now because the true acid has been neutralized, and that now shown is due to acid salts only.

Ex. Suppose the first estimation gave a total acidity of 0.274 per cent., and the second one of 0.053. The difference, or 0.211, would be the percentage of hydrochloric acid present, and 0.053 would be that of acid salts. Were no difference observed between the first and second estimation, this would show that there was no hydrochloric acid present at all. If the second estimation gave no acidity at all, it would prove that there were no acid salts in the fluid.

Organic acids, *e.g.* lactic and volatile acids, can also neutralize calcium carbonate, so if the qualitative tests reveal their presence they must first be removed, the former by extraction with ether, the latter by means of distillation.

(2) *Toepfer's method.* (a) First estimate the total acidity in the usual way with $\frac{N}{10}$ caustic soda, using phenolphthalein as an indicator, and noting the result.

(b) Take 5 c.cm. of the filtrated contents and add as the indicator one drop of a 0.5 per cent. solution of di-methyl-amido-azo-benzol. A bright red colour is produced with the free hydrochloric acid (see p. 151); if this fluid be now titrated with $\frac{N}{10}$ caustic soda, the red colour will go when all the free hydrochloric acid is neutralized, and will be replaced by a yellow tint. Note the result, which gives the amount of free hydrochloric acid present.

(c) Take 10 c.cm. of the gastric fluid and add a drop or two of 1 per cent. solution of sodium-alizarin-sulphonate as indicator. A brown colour results; dilute the liquid

with a little water. Now titrate once again in the usual way, when a beautiful purple colour is developed, going at first on shaking, but afterwards becoming permanent. This purple colour results from the neutralization of all the elements causing acidity, except the combined hydrochloric acid. Therefore, the result of this titration (which represents free hydrochloric acid, organic acids, and acid salts), subtracted from the total acidity, gives the amount of combined hydrochloric acid. In order to recognize the purple colour when it appears, it is as well to have prepared, for the sake of comparison, a little 1 per cent. solution of caustic soda, to which three or four drops of the alizarin solution have been added.

General results of this method. First titration gives the total acidity: call this A; second titration gives the free hydrochloric acid: call this B; third titration gives combined hydrochloric acid: call this C; and since $A - B =$ combined HCl, organic acids, and acid salts, then $(A - B) - C =$ organic acids and acid salts.

Acid salts. Those present in gastric fluids are usually acid phosphates, generally the sodium salt. This is regarded by some physiologists as taking a part in the production of hydrochloric acid in the stomach from sodium chloride, thus: $\text{NaH}_2\text{PO}_4 + \text{NaCl} = \text{HCl} + \text{Na}_2\text{HPO}_4$. The di-sodic hydrogen phosphate being absorbed increases the alkalinity of the blood during digestion, and, secondarily, that of the urine.

CHAPTER II.

Gastric fluids concluded. Lactic acid—Volatile organic acids—Acetic acid—Butyric acid—Peptones and albumoses—Sugar—Starch—Pepsin—Rennet-ferment—Blood—Bile—Ammonium carbonate—Acetone—Gases in the stomach—Absorptive power of stomach—Motor power. Bibliography.

Lactic acid ($C_3H_6O_3$). This is an oxy-derivative of a fatty acid, propionic acid, as will be gathered from its formula, $C_2H_4 < \begin{matrix} OH \\ CO_2H \end{matrix}$. It has already been pointed out that it may occur in the early stages of normal digestion when carbohydrates have formed part of the meal, but it is to be regarded as a pathological constituent if it be found several hours after food or between meals. It is notably present in cases of gastric dilatation with fermentation of the contents.

Being non-volatile, it is not driven off if the fluid containing it be heated to 100° C., and it cannot be detected by its odour like butyric acid.

Tests for lactic acid. In some cases these are applied to the stomach contents as they are, in other cases the acid must first be extracted with ether. This is done by shaking up a tolerably abundant quantity of the gastric

fluid with 25 c.cm. of ether in a cylindrical vessel. After standing, the ether is removed by a pipette and kept, and the process may be repeated with a second supply of ether. The ethereal extracts are mixed, evaporated to dryness, and the residue dissolved in a little water. To this solution the tests may be applied.

(1) *Uffelmann's test.* The reagent used consists of a 20 per cent. solution of crystals of carbolic acid in water, a drachm of which, when used for testing, is diluted with twice its volume of water, and has added to it a drop or two of fairly strong solution of perchloride of iron. There results from this an amethyst blue fluid. For a test it is sufficient to prepare 10 c.cm. at a time. This quantity having been placed in a test-tube, a little of the suspected fluid is allowed to run down the inside of the tube, when, if lactic acid is present, a canary-yellow coloration is produced. Hydrochloric acid and volatile acids, such as acetic, simply cause the blue colour to disappear. The test is delicate, but acid salts and peptones are apt to exercise a disturbing influence.

(2) *Perchloride of iron test.* This is very simply performed with a solution of ferric perchloride in water, so weak as to be almost colourless. If a fluid containing lactic acid be added, the previously faintly-tinted liquid will become distinctly yellow. A contrast tube of solution should always be employed. This test is as delicate and reliable as it is simple, and is apparently unaffected by other constituents of the stomach contents.

(3) *Boas's aldehyde test.* Boas¹ has introduced a test based on the production of aldehyde from lactic acid, and on its

¹ *Deut. med. Wochens.*, No. 19, 1893.

reaction to an alkaline iodine solution, producing iodoform. It is, however, too complicated for clinical work.

The quantitative estimation of lactic acid alone may be made by thoroughly extracting it from the gastric contents by means of repeated shakings with ether, evaporating the extract to dryness, dissolving the residue in distilled water, and titrating in the usual way with $\frac{N}{10}$ solution of caustic soda.

Quantitative estimation of organic acids generally. If it be desired to make a quantitative estimation of the organic acids generally without differentiating them, the most useful process for clinical work is that devised by Hehner and Seemann.

It is based on the fact that organic salts, on incineration, become converted into carbonates, and is performed as follows :

First, take the total acidity in the usual way with 5 c.cm. of the gastric fluid and note the result. When the point of neutralization is reached, there are no longer acids in the liquid tested but chloride, and, let us say, lactate and butyrate of sodium (provided hydrochloric, lactic, and butyric acids were present at first). The liquid is now evaporated on a water-bath, and the dry residue incinerated, whereby the organic sodium salts are converted into carbonates, the chloride remaining as it was. The dry and burnt residue is thoroughly mixed with a little distilled water, and then as much $\frac{N}{10}$ solution of hydrochloric acid is added as was used of $\frac{N}{10}$ soda at first. Part of this hydrochloric acid will be neutralized by the carbonates that

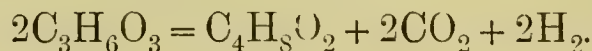
resulted from the combustion of the organic salts, converting them into chlorides, while part will remain unaffected, as it will not act on the sodium chloride present which resulted from the neutralization of the hydrochloric acid originally present.

It is only necessary now to titrate the liquid with $\frac{N}{10}$ soda solution to ascertain how much of this hydrochloric acid remains free, and this corresponds to the hydrochloric acid originally present. The difference between the first and the last titration gives the acidity due to organic acids.

Ex. Suppose the first analysis gave an acidity of 0.387 per cent., and the last one of 0.251; this latter figure would indicate the amount of hydrochloric acid present, while the amount of organic acids would be $0.387 - 0.251$, or 0.136 per cent.

Volatile organic acids. These generally consist of either acetic acid ($C_2H_4O_2$) or of butyric acid ($C_4H_8O_2$), or of both. They are acids of the fatty series, and are always to be regarded as abnormal elements in gastric fluids, arising from stagnation and fermentation of the stomach contents.

Acetic acid arises from the fermentation of glucose and maltose, while butyric acid is produced by the action of various bacteria on carbohydrates, notably by the bacillus butyricus. It may also develop from lactic acid thus:



Tests for acetic and butyric acids. The presence of these acids may be suspected from the odour of the stomach contents, acetic acid having a vinous smell while that of butyric acid is rancid.

To apply chemical tests to them successfully it is necessary first to separate them from the stomach contents, and to do this we may take advantage either of their volatility and distil them off, or of their solubility in ether and extract them in this menstruum.

(1) *Separation by distillation.* This is most thoroughly accomplished by using a flask attached to a proper condenser, such as Liebig's, but the author has often performed it satisfactorily by using a small distillation flask, the projecting arm of which is inserted into a long narrow test-tube of the same length. This tube should be kept quite cool inside a flask of cold water. On applying moderate heat, a sufficient quantity of distillate is obtained in ten minutes. Lactic and hydrochloric acid do not pass over. By testing the distillate with litmus paper we may assure ourselves, in the first place, whether any volatile acids are present or not.

(2) *Extraction with ether.* This is performed, as already described on p. 155, by shaking up 50 c.cm. or more of the gastric contents with two different supplies of ether and evaporating the ethereal extracts, only in this case the latter process must be conducted at room temperature lest the volatile acids be driven off. The dry residue is dissolved in a little water, and the acids, if present, may often be recognized by their smell. The reaction to litmus paper, although positive, will not be conclusive of their presence, as lactic acid is also soluble in ether.

Acetic acid is recognized by taking a little of the fluid, neutralizing it exactly with sodium carbonate, and adding a drop or two of dilute solution of ferric perchloride.

The appearance of a red colour, due to ferric acetate, proclaims the presence of acetic acid.

Butyric acid may be recognized in the first place by taking a little of the fluid in a test-tube, adding a drop or two of strong sulphuric acid and a little absolute alcohol and boiling, when butyric ether will form and present the agreeable odour of "pine-apple rum."

If, secondly, a little of the liquid be poured into a watch-glass, and a few fragments of calcium chloride be added, the acid will separate out and form oily droplets on the surface, as it is insoluble in the presence of calcium salts.

Quantitative estimation of volatile acids alone. A rough approximation may be made thus: First take the acidity of the liquid, then measure out, say, 50 c.cm., evaporate to a small bulk, add a little water, evaporate again, and finally, when cool, make up to the original volume with distilled water. The difference between the acidity taken now and that obtained at first will be due to the acids driven off by heat, *i.e.* to the volatile acids.

Albumoses and peptones. These are practically invariably present after a test-meal, and may be easily recognized by adding a drop of 1 per cent. cupric sulphate solution to 5 c.cm. of the gastric fluid and then excess of caustic potash. The development of a rose-pink colour (biuret reaction) indicates the presence of peptones and albumoses. Differential tests between them, and between the principal albumoses, will be found in the chapter dealing with proteid bodies in urine (see p. 72). As mentioned above, it is rare to find no products of peptic digestion in the stomach after food, but this may occur

temporarily in acute gastric catarrh and acute gastritis where no hydrochloric acid or pepsin seems to be secreted at all.

Starch and sugar. Under the influence of the ptyalin in saliva, the starch taken in food is converted largely into maltose, and in that form, along with, perhaps, some achroodextrin and erythrodextrin, enters the stomach. Some of it may even find its way there as unchanged starch, and for a short time undergo conversion into maltose (see p. 146). Very frequently no starch is found in the stomach one hour after the ingestion of farinaceous food, but if any be present it may be recognized by the fact that it strikes a blue colour with tincture of iodine. This reagent gives a red coloration with erythrodextrin and none at all with achroodextrin. The microscope will often reveal starch granules, recognizable by their large size, oval shape, and concentric markings.

Sugar is readily detected by its reducing effect on Fehling's or Trommer's solution; it is usually present as maltose, with occasionally traces of glucose. After a meal containing starchy foods, sugar is almost invariably present in the stomach contents.

Pepsin. The activity of pepsin in the gastric juice may be tested by its proteolyzing effect on some albuminous substance.

(1) *White of egg test.* To perform this we may employ either a solution of white of egg in water, or discs of the same cut out of hard-boiled egg, 1 mm. in thickness and 1 cm. in diameter.

The solution of white of egg is made by breaking the white of one egg into a cup, clipping it well with scissors

to divide the fibrous partitions, and then shaking thoroughly in a flask with 100 c.cm. of water. The more solid particles are allowed to subside and 10 c.cm. of the clear fluid are placed in a test-tube and thoroughly coagulated by boiling. Either to this when cool, or to a disc of white of egg, are added 20 c.cm. of filtered gastric contents, a little water, and a drop or two of acid. hydrochloric. dil. (B.P.). A control tube is prepared in a similar way, but the stomach fluid is first boiled to kill the pepsin-ferment. Both tubes are now placed in a warm chamber or water-bath at 37° C. for one hour, and examined at the end of that time.

If the pepsin be active, the tube in which it was present unboiled will be quite, or almost quite, clear, the other remaining opaque.

(2) *Grützner's carmine-fibrin test.* Some fibrin obtained by whipping fresh ox-blood is thoroughly washed with water and allowed to soak for twenty-four hours in a 0.25 per cent. solution of carmine in water containing a little ammonia. At the end of that time it is removed and washed with water till no more of the dye comes away. The shreds of fibrin remain of a deep red colour and retain the carmine so firmly that not even dilute hydrochloric acid will set it free.

If, however, a shred or two be placed in a test-tube with a little of the filtered gastric juice and a few drops of weak hydrochloric acid, and be kept at a temperature of 37° C., in a very few minutes the fluid will become red from the carmine set free under the peptonizing influence of the pepsin. The depth of colour obtained in a given time will be a measure of the digestive activity. The

carmine fibrin may be preserved in glycerine ready for use for an almost indefinite time.

Pepsin is rarely absent from gastric juice, except in marked gastritis atrophicans.

The rennet-ferment. It must not be forgotten that the gastric juice contains another ferment, viz. rennet, which has the power of coagulating milk even in a neutral medium. It is usually present in health, but may be absent in cancer, and other serious affections of the stomach.

Test for rennet. Take 5 c.cm. of fresh cow's milk, neutral in reaction, and boil. Add to this when cool, 10 c.cm. of the filtered gastric juice which has been exactly neutralized, and place the mixture in a warm chamber for half an hour. At the end of that time, if active rennet be present, particles of coagulated casein will be found in the tube.

Blood. This is an abnormal constituent of the stomach contents, and is found typically in gastric ulcer and cancer of the stomach, as well as in pin-hole erosions of gastric arteries; it appears too in venous congestion secondary to portal obstruction and organic heart disease. It may also occur as the result of excessive straining and vomiting, or may find its way into the stomach from the œsophagus or duodenum as a result of lesions there. Not unfrequently the blood is swallowed in cases of epistaxis, hæmoptysis, and bleeding from the gums. In appearance it may be florid, or dark red, or so altered as to be brown or even black, the condition known as coffee grounds' material; in the latter case part of the blood is always present as hæmatin.

Tests for blood. (1) *The guaiac and ozonic ether test* may be applied here as in other conditions (see p. 98). This

test is not always reliable, as the stomach may contain other substances besides blood capable of giving a blue colour with guaiac.

(2) *Hæmin crystals*. A particle of the blood may be dried on a slide, a crystal of sodium chloride and some glacial acetic acid added, and the whole heated, when hæmin crystals will form as described on p. 99.

(3) *Formation of Prussian blue*. Since both hæmoglobin and hæmatin contain iron, this may be utilized as a test for blood. A little of the suspected substance is heated in a small porcelain crucible with a crystal of potassium chlorate and a drop or two of strong hydrochloric acid. The fluid becomes yellow from the formation of perchloric acid, and the iron is oxidized into ferric chloride. On cooling, a little water is added and a few drops of 5 per cent. solution of potassium ferrocyanide, when a formation of Prussian blue takes place. This is a fairly good test if the blood be not too scanty, but the observer must make sure first that the patient is taking no preparation containing iron.

Bile. The presence of bile in vomited matter is often clearly revealed by its green colour, due to biliverdin, which is always present. It is an indication of regurgitation into the stomach from the intestines.

Test for bile. Filter some of the gastric contents, and over the green residue on the filter-paper run a little hot alcohol acidulated with sulphuric acid; this, as it comes through, may be caught in a test-tube, and will be found to be of a green colour. Gmelin's test may also be applied (see p. 102). Bile-acids are usually present at the same time.

Ammonium carbonate. This salt is sometimes found in considerable quantities in the stomach, and is held by some authorities to indicate a retention and decomposition of urea within the system.

Test. Mix some of the gastric fluid with either a little milk of lime or caustic soda in a beaker, and cover the latter with a watch-glass to the under surface of which a strip of moistened red litmus paper has been made to adhere. The liberation of the ammonia by the fixed alkali will quickly turn the test paper blue, and the gas may also be recognized by its smell when the watch-glass is removed. This reaction will of course be given by any ammonium salt.

Acetone. This body has from time to time been detected in the stomach contents. It does not seem to have any etiological relationship to gastric disorder, but appears as a result of some disturbance of proteid metabolism within the body.

It may be detected by distilling the stomach contents after the addition of a little phosphoric acid, and testing the distillate by Lieben's reaction (see p. 92). The author has found it in this way in a few cases of dilatation of the stomach.

Gases in the stomach. Many patients with gastric disorders suffer much from flatulence due to the following gases: oxygen, nitrogen and carbonic acid gas swallowed with saliva, carbonic acid and hydrogen gas developed in the stomach (see p. 158), and occasionally even sulphuretted hydrogen. It is not necessary and hardly practicable to apply tests for these clinically.

Absorptive power of the stomach. To test this the

method recommended by Penzoldt may be employed. It is performed by making the patient swallow a capsule containing 15 grs. (or 1 grm.) of potassium iodide. This drug after absorption quickly appears in the saliva, and may be detected there by touching a piece of starch test paper moistened with fuming nitric acid with a drop of saliva every half minute. The appearance of a dark blue stain indicates the presence of the iodide, and is commonly seen in ten minutes. Absorption appears to be delayed in certain diseases of the stomach.

Motor power. Ewald has suggested as a test for this that the patient should swallow a capsule containing one grm. of salol, and that the urine should be tested by means of ferric perchloride for the first appearance of salicylic acid. As salol does not decompose into salicylic and carbolic acids till it reaches the intestine, it is obvious that the colour in the urine will not show till the motor power of the stomach has passed the drug into the duodenum. The test usually answers positively in about 45 minutes. The motor power is markedly deficient in dilatation of the organ and in chronic catarrh.

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SECTION III.

THE SALIVA AND SPUTUM.

The Saliva—General appearance—Quantity—Reaction—Microscopical characters—Chemical composition—Mucin—Ptyalin—Globulin—Sulphocyanide of potassium. The Sputum—General appearance—Tubercle bacillus—Pneumococcus—Elastic tissue—Curschmann's spirals—Charcot-Leyden crystals—Bronchial casts—Other elements in the buccal cavity—Diphtheria bacillus—Leptothrix—Thrush—Actinomyces. Bibliography.

I. **The saliva.** The saliva is not a fluid which, as a rule, is subjected to chemical examination for clinical purposes, because it has not yet proved itself of much value for diagnostic ends. Still it is advisable that the student should know the chief reactions it shows, and be able at least to distinguish it when returned, for example, from an œsophageal diverticulum, from gastric fluid really vomited.

Before collecting saliva for examination, the patient should be made to rinse out the mouth thoroughly with water containing a little bicarbonate of sodium in solution.

(1) *General appearance.* Saliva generally appears as a

semi-transparent opalescent fluid, usually mixed with air bubbles when poured from the mouth. It is often mucoid in character from the mucin it always contains. If some be collected from the mouth in a conical glass, it will be found, after the lapse of a short time, that the lower part of the fluid looks cloudy and turbid; this is due to the precipitation of calcium carbonate. Mixed with saliva may be foreign particles chiefly in the form of food that has lodged about the teeth, while the normal colour may be reddened by the presence of blood or rendered brown where the patient has been chewing tobacco.

(2) *Quantity.* Normally 2-3 pints of saliva are secreted in twenty-four hours. When distinctly increased we deal with the condition known as "ptyalism," which is met with in nervous and mental diseases, in rabies, sometimes in infective fevers and during the early months of pregnancy; it occurs too after the prolonged use of mercury and its compounds (the most common cause of it), and of iodine, salts, pilocarpine, tobacco, etc. The flow is diminished in inflammation of the salivary glands, during severe fevers, and in atropine poisoning. When very scanty, apart from other morbid conditions, it gives rise to the affection named Xerostoma.

(3) *Reaction and specific gravity.* The saliva itself is always alkaline in health, as may be shown by its effect on a piece of red litmus paper. If, however, a piece of blue litmus paper be placed under the tongue, it will be found in most persons to exhibit the acid reaction. This is due probably to acids developed by bacterial action on particles of food adhering to the teeth. The saliva itself has been found

to be acid occasionally in diabetes mellitus, in fever (especially rheumatic), and in dyspepsia. The specific gravity of saliva is between 1.002 and 1.006.

(4) *Microscopical characters.* Under the microscope one usually finds granular salivary corpuscles, rather larger than leucocytes, and squamous nucleated epithelial cells derived from the hard palate.

(5) *Chemical composition.* Halliburton¹ gives the following as the solid constituents of saliva: *Organic*—Mucin, ptyalin, proteid (a globulin), sulphocyanide of potassium. *Inorganic*—Sodium chloride (most abundant salt), sodium carbonate, calcium carbonate and phosphate, magnesium phosphate and potassium chloride.

Pathologically, urea has been detected in saliva in cases of nephritis, but sugar is not present in diabetes. Drugs swallowed are often excreted into the mouth by the salivary glands, notably iodides and bromides.

Mucin. (a) A little saliva run into a beaker of clear distilled water causes a cloudiness due to mucin being precipitated.

(b) The addition of a little acetic acid to saliva causes a flaky precipitate of mucin.

(c) Mucin is soluble in dilute alkalies like lime-water.

Ptyalin. This ferment is the most important constituent of saliva, and may be tested for as follows: Make a little weak starch solution with boiling water; when cool place a few c.cm. in a test-tube with some saliva. Set the tube in a beaker of warm water for a few minutes, and on testing the fluid at the end of that time by means of Trommer's or Fehling's test, it will be found

¹ *Essentials of Chemical Physiology*, 1899, p. 44.

that the starch has become almost wholly converted into maltose, a little perhaps remaining as dextrin. This property of saliva may be taken advantage of to distinguish it from other watery fluids from the stomach, mouth, or gullet. Saliva acts best at body-temperature in a neutral or faintly alkaline medium. If the ferment happen to be destroyed, the fact of the fluid containing little proteid matter, and having a low specific gravity, suggests saliva.

Globulin body. Filter some saliva, and to the filtrate add a few drops of nitric acid ; a precipitate forms which turns yellow on boiling (xantho-proteic reaction. See p. 62).

Sulphocyanide of potassium. The addition of a little dilute solution of ferric perchloride to saliva will show a reddish tint, due to the formation of sulphocyanide of iron. This colour is not affected by heating, or by the addition of acids, but is discharged by mercuric chloride.

II. The sputum. **General appearance of sputum.** The sputum should be collected in a glass spittoon-jar, or in one of metal enamelled white. The quantity varies very greatly, and is not a criterion of the severity of the disease. The reaction is usually alkaline. Careful attention should be paid to the naked-eye appearance, colour, smell, viscosity, etc. The following are the chief varieties of sputum :

(1) *Frothy and watery*, resembling saliva ; met with in acute bronchitis and pulmonary oedema.

(2) *Muco-purulent* and *purulent*, often thick, and even tenacious ; sometimes containing round somewhat flattened greenish-white masses (nummular sputum). This variety

is met with in chronic bronchitis and chronic catarrh of the bronchial tubes, in phthisis, in bronchiectasis (then very foetid), in discharging empyema, or in pulmonary abscess.

(3) *Prune-juice sputum*, thin and watery in œdema of the lung, tenacious and viscid in the early stages of pneumonia.

(4) *Yellow or green sputum*, where bile becomes mixed with it, as in the case of rupture of a hepatic abscess into the thorax.

(5) *Bloodstained and florid sputum*, in cases where there is intra-pulmonary bleeding. Error often arises here from blood derived from the pharynx, nose, or stomach becoming mixed with the expectorated material.

(6) *Black sputum*, met with in the cases of coal-miners and others working in an atmosphere heavily laden with carbon particles.

(7) *Red currant jelly sputum*, said to be specially associated with malignant disease of the lungs.

The microscopic study of the sputum is of much greater importance than the application of chemical tests, and will therefore be dealt with first.

It must always be borne in mind that the sputum is very apt to be contaminated with foreign matter, such as particles of food, fragments of tobacco, etc., and the student must be on his guard against error arising therefrom.

Tubercle bacilli. It is unnecessary here to insist on the importance of the discovery of the bacillus tuberculosis in the sputum in cases of pulmonary disease. The finding of the organism is a positive proof of the

tubercular element in an illness, but great caution must be exercised in drawing the opposite conclusion when the bacillus is *not* found. One must not rest content in such a case with less than several careful examinations.

Morphologically the tubercle bacillus has the form of a slender rod, which may be curved or bent a little; its length on an average is from $2.5-3.5\mu$, or from one-third to one-half the diameter of a red blood cell. The fact that it takes up basic aniline stains and retains them tenaciously enables us to demonstrate it with tolerable ease.

Staining for tubercle bacilli. It is first of all necessary to select a suitable portion of sputum and make a film of it. To do this, pour some of the sputum into a flat glass dish placed on a black ground, and pick out by means of a handled needle a likely piece of the material. A little caseous mass or a small muco-purulent shred often yields excellent results. The piece is lifted out by means of the handled needle and a pair of fine forceps and placed on a perfectly clean square cover-glass (No. 2); a similar cover-glass is now dropped on the top of it, and the tiny mass flattened out between them to make a film. The two slips are then made to slide apart, and dried in the air, which they usually do quickly.

The dry film must now be fixed, which is accomplished by passing the cover-glass, held in a pair of forceps, through the flame of a bunsen burner or spirit lamp two or three times. The glass should pass through the flame pretty quickly. In this way the albuminous matters in the sputum are made to coagulate by the heat, and the film caused to adhere firmly. In this as in all

other manipulations with cover-glasses, a pair of Cornet's forceps will be found very useful, as by cross action they continue to grasp the cover-glass firmly even when left alone. Staining is usually carried out by the Ziehl-Neelsen method with carbol fuchsin. A watch-glass or small porcelain capsule is filled with the stain, which has the following composition :

Basic fuchsin, -	-	-	-	-	-	-	1 part.
Absolute alcohol, -	-	-	-	-	-	-	10 parts.
Solution of carbolic acid (1-20),	-	-	-	-	-	-	100 parts.

If the stain has been made up for a little time it should be filtered before use.

The dish containing the stain is supported on a simple wire frame placed on a ring of a retort stand, and the cover-glass is floated in it film downwards. At the same time heat is applied to the dish by means of a spirit lamp till steam begins to rise. The flame may be withdrawn then, and re-applied for a few seconds at intervals of half a minute. At the end of five minutes the staining is complete. The cover-glass is removed from the stain, washed well in water, and then placed for two to three minutes in 20 per cent. sulphuric or nitric acid to decolorize it. The film now presents a yellowish tint, and is washed once more in water, when, if decolorization be complete, it will have merely a pink tint.

In order to counter-stain the film for the sake of contrast, it should be floated on a saturated watery solution of methylene-blue for thirty seconds. It is then once more washed in water, dried between two layers of filter-paper, mounted in xylol balsam, and examined. If desired, Gabet's modification may be used where the

decolorizing and counter-staining are effected simultaneously by placing the fuchsin-stained films in a solution composed of methylene-blue, 2 parts; sulphuric acid, (25 per cent.), 100 parts. As a rule, however, the film is first decolorized by acid alone, and then counter-stained.

In examining, where possible, an oil immersion lens ($\frac{1}{12}$ in.) should be used, but the organisms can be made out with a D Zeiss objective and No. 3 eye-piece. In using the oil immersion a drop of pure cedar oil should be placed on the cover-glass and the lens lowered into this by means of the hand. The fine adjustment is then used for focussing, and an Abbe's condenser is essential. After use the lens should be drawn up out of the oil and wiped dry with an old silk handkerchief.

The slender, straight, or curved bacilli appear red on a blue background. They may be arranged in groups or lines, or in couples forming an obtuse angle. The blue background consists of pus and epithelial cells, particles of food, etc.

Fraenkel's pneumococcus (*diplococcus lanceolatus*). This organism occurs in most cases of pneumonia whatever be the clinical variety, and is now regarded as being definitely associated with the disease. It occurs as small oval cocci, $1\ \mu$ in length, and arranged in pairs or in lines. It may be obtained for examination post mortem from the fresh lung, at an early stage of pneumonia, or during life from the gelatinous part of rusty pneumonic sputum.

Films are prepared in the usual way, and staining may be carried out by the Ziehl-Neelsen method as

described above, the results so obtained being often quite satisfactory. Muir and Ritchie¹ advise staining for only a few seconds, or else over-staining with subsequent decolorization with alcohol till the preparation is just tinted. Gram's method also gives good results, and the following modification of it has been used with advantage.

Gram's method. The solutions required are (a) a saturated solution of gentian violet in water; (b) a 20 per cent. aqueous solution of carbolic acid; when staining is to be carried out, one part of (a) is mixed with ten parts of (b), and the whole filtered. In this fresh mixture the film is stained for five minutes, thereafter washed in water, and then placed in Gram's solution, the composition of which is as follows:

Iodine, - - - - -	1	gram.
Potassium iodide, - - - - -	2	grms.
Distilled water, - - - - -	300	c.cm.

In this the film is allowed to stain till it assumes a purple-black colour; this usually requires from half to one minute. The cover-glass is now removed from the solution, washed with alcohol or methylated spirit, and decolorized by means of clove oil till the colour is nearly all gone. The oil is removed by further treatment with alcohol, the film washed in water and finally stained for two to three minutes in a saturated solution of Bismarck brown in equal parts of alcohol and water, which makes a good counter-stain. The specimen may now be dried between filter paper, mounted in xylol balsam, and examined.

By Gram's method the following organisms can be stained: Fraenkel's pneumococcus, bacillus of anthrax, of

¹ *Manual of Bacteriology*, 1897, p. 188.

diphtheria, and of tetanus, streptococci and staphylococci, while the bacilli of tubercle and of leprosy stain, but not very well.

Friedländer's pneumo-bacillus, the bacillus coli communis, as well as those of typhoid, glanders, and influenza, and the gonococcus, are decolorized by this method.

Elastic fibres in sputum. These are generally derived from the bronchi or alveoli, but may take origin from the



FIG. 21.--ELASTIC FIBRES OF LUNG TISSUE. (Finlayson.)

walls of the arteries. Their presence indicates a breaking down of tissue, and thus constitutes a sign of positive value in doubtful tubercular cases. It must, however, be borne in mind that they may occur in any necrotic process affecting lung tissue, and may thus be met with in gangrene, abscess, etc.

To examine for them, we may simply pick out a likely particle of sputum, lay it on a slide, and press a cover-glass gently but firmly upon it to flatten it out. The microscope may then reveal the presence of the fibres.

If that fail, it is advisable to boil some of the sputum with caustic potash to thin and clarify it. To do this, take, say, 20 c.cm. of sputum and place it with an equal quantity of 10 per cent. caustic potash solution in a porcelain basin; it may be necessary to dilute the mixture with a little water. Bring now to the boil over a spirit lamp, with constant stirring, and after a couple of minutes' boiling pour off into a conical glass. The elastic fibres gradually sink as a sediment, of which a little may be removed after several hours for microscopic examination.

In appearance they have the form of several fibres placed side by side (fig. 21), finally separating from one another with free curling ends, or they may be arranged so as to enclose irregular spaces, this being indicative of elastic tissue derived from alveoli.

Curschmann's spirals. Curschmann's spirals are especially associated with bronchial asthma, and are more

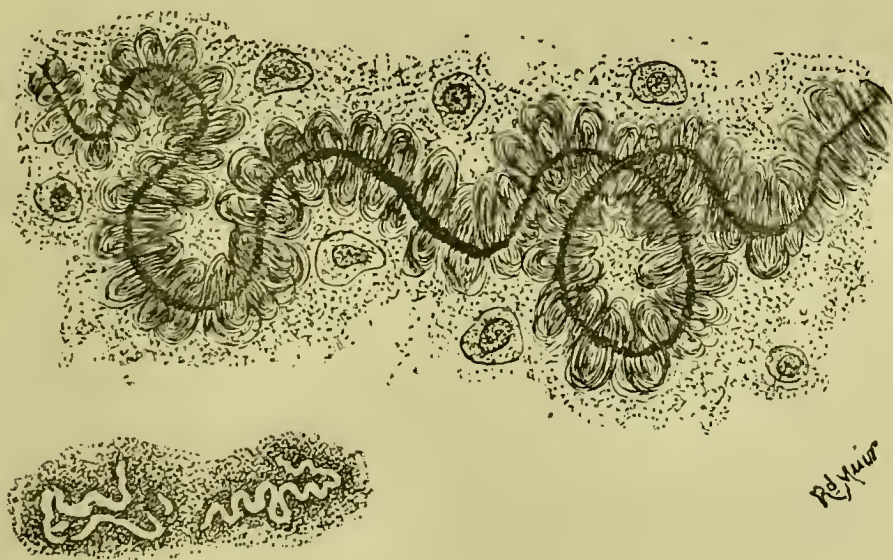


FIG. 22.—CURSCHMANN'S SPIRALS IN SPUTUM, $\times 200$ AND NATURAL SIZE. (Hutchison and Rainy.)

abundant in the sputum in the early stage of an attack,

being sometimes in large numbers. To the naked eye they present the appearance of small semi-translucent balls in the expectoration. If some of this material be pressed flat between two glass plates and examined over a dark ground, the small spirals themselves may be seen by the naked eye, in length 1 cm., more or less (fig. 22).

Examined between two glass slides under a low power, their form can be distinguished more clearly. They have sometimes the appearance of dull coils, but in others in the axis of the spiral there appears a bright clear line formed of mucin. The spirals themselves are composed of mucin, and take origin in the fine bronchioles. A higher power may show many leucocytes entangled in the coils, as well as the crystals to be described next.

Charcot-Leyden crystals. These are elongated, colourless, octahedral crystals (fig. 23), which not infrequently

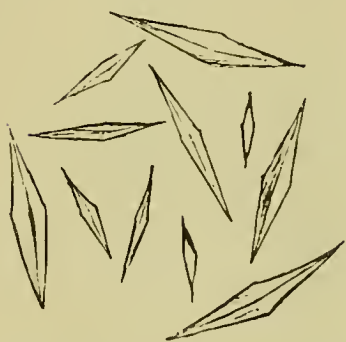


FIG. 23.—CHARCOT-LEYDEN CRYSTALS.

occur in the expectoration in cases of bronchial asthma. They are found, however, in spermatic fluid, fæces, and leukæmic blood as well.

At one time they were considered to have a distinct etiological relationship to asthma, it being supposed that they irritated the nerve-endings and set up the paroxysms in this way. This view is now discarded. Chemically they appear to be of the nature of a phosphate of an organic base, to which the formula C_2H_5N has been given, and they probably take origin in the stagnated secretion of the smaller bronchioles. As already

mentioned, they may adhere to Curschmann's spirals, giving them a peculiar bristling appearance.

Bronchial casts, etc. These occur in expectorated matter from time to time. They are composed of fibrin exuded into the fine twigs of the terminal bronchioles, whose shape they retain. They are found in pneumonia, and more extensively in chronic bronchitis. In addition to these, there may occur in sputum pus corpuscles, red and white blood cells, fatty crystals, crystals of hæmatoidin, and other formed elements, for descriptions of which the student is referred to larger text-books.

Chemical examination of sputum. The application of chemical tests to sputum has a limited range, and is of little clinical significance. Various proteid substances occur in expectoration, notably serum-albumin, mucin, and occasionally peptone in cases where pus occurs. Volatile fatty acids may be present in cases where there is long-retained secretion or necrotic processes, as in bronchiectasis and gangrene of the lung. Halliburton¹ gives some analysis of the sputum in cases of lung disease, from which it would appear that the expectoration in pneumonia is especially rich in sodium chloride, proteids, and extractives.

Other elements in the buccal cavity. *Membrane and bacillus of diphtheria.* In cases of suspected diphtheria, a small piece of the membranous patch should be removed with a pair of forceps, teased out, and rubbed over a cover-glass to form a film. If it be very dry, it may first be moistened with a drop of distilled water. The film is allowed to dry, then stained for two or three minutes

¹ *Chemical Physiology and Pathology*, 1891, p. 448.

in a saturated aqueous solution of methylene-blue, washed, dried, mounted, and examined for the Klebs-Loeffler bacillus.

The organism is about $6\ \mu$ in length, and a fifth of that broad. It stains well with methylene-blue, and often irregularly, so that the protoplasm seems beaded. The ends may be slightly swollen, staining darker than the rest of the microbe, and in some cases are actually clubbed. It may also occur in a much longer form. The bacilli may lie in lines, or be arranged in angular figures.

Mixed with them may be staphylococci, streptococci, and the pseudo-diphtheria bacillus described by Loeffler and by Hofmann. Sometimes the true organism occurs in large numbers, or it may be very scanty.

By this means it is possible to make a correct diagnosis without having recourse to cultural methods, in a certain proportion of cases. It must always be borne in mind that the Klebs-Loeffler bacillus may persist in the throat for weeks or even months after the person has been apparently cured, and that it may even be found in those who appear to have nothing the matter with them, but who have been in attendance on diphtheritic patients. Success in making a diagnosis by microscopical examination of the membrane depends obviously to a very great degree upon the experience of the observer, and on his knowledge of the characters of the true bacillus.

Where doubt exists, cultures should be made, and to this end a small portion of the membrane must be removed by a swab and sent to a laboratory for inoculation on Loeffler's blood-serum. The swabs consist of pieces of stout wire, say 18 cm. (7 in.) long, with a piece

of absorbent cotton wool twisted on one end. Each swab is placed in a clean test-tube a little shorter than it is, plugged in with more wool, and then the whole is sterilized by heat. When about to use, withdraw the plug and the swab, apply the latter to the false membrane with a twisting motion, and then replace in the tube.

Cultures of Klebs-Loeffler bacillus grow in eighteen hours, and at the end of twenty-four show as distinct little masses as large as a pin's head.

Leptothrix buccalis. Among the tartar at the roots of the teeth, and in the carious dentine of decaying teeth, may be found numbers of long segmented bacilli arranged in ribbon-like bundles. They are termed leptothrix. When treated with a watery solution of iodine in iodide of potassium, the bacilli stain bluish-red. They are non-pathogenic, but probably aid in bringing about the decay of the teeth.



FIG. 24.—*a*, SCRAPING FROM A PATCH OF THRUSH $\times 800$; *b*, CULTURE OF *SACCHAROMYCES ALBICANS* (*MONILIA CANDIDA*) $\times 800$. (Hutchison and Rainy.)

Thrush or Muguet. The white or whitish-grey membrane in this affection is the habitat of a fungus—the *saccharomyces albicans* (*oidium albicans*, Robin), described in more modern times as the *monilia albicans*. It forms a branching ribbon-like structure composed of long segments, and in the midst of it may be seen the free-lying spores or gonidia (fig. 24).

Actinomyces or ray-fungus. Since actinomycosis may affect the tongue or the jaw, and also occurs in pulmonary tissue, the fungus may be found in pus from the buccal cavity, or expectorated from the lung. It appears to the naked eye in the form of small white or yellow masses the size of a pin's head. The fungus itself is a pleomorphous organism belonging to the streptothrix group, and reveals three forms under the microscope (Muir and Ritchie):

1. Thin filaments $5\ \mu$ in diameter, often interlaced.
2. Cocci—the protoplasm of the filaments broken up into little round bodies, sometimes lying free.
3. Clubs—elongated pear-shaped bodies, homogeneous in structure, and forming a radiating mass. They are often absent in man, and are very fragile.

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SECTION IV.

THE BLOOD.

CHAPTER I.

Method of procuring for examination — Colour — Reaction — Specific gravity—Coagulability—Iso-tonic coefficient—Estimation of hæmoglobin—Enumeration of red corpuscles.

THE clinical examination of the blood has of late years assumed great importance, especially with regard to the leucocytic elements in it, and the variation of these cells both in number and in variety under various morbid conditions. As there are few, strictly speaking, chemical processes to which the blood can be subjected clinically, reliance is placed chiefly on physical and microscopical methods in its examination.

Blood is usually removed for the purposes of clinical work from the lobe of the ear or the tip of the fore finger (preferably from the dorsal aspect near the root of the nail). A suitable instrument for the purpose is a sharp narrow lancet or triangular surgical needle, which may be passed through a disc of cork, so that only one-eighth of an inch is exposed for the purpose of puncturing. Care

must always be taken that the instrument used is quite clean.

Suppose the lobe of the ear has been selected; the skin is first washed with a piece of clean lint dipped in water, and then thoroughly dried. The operator having rubbed the lobe briskly to make it hyperæmic, seizes it between the forefinger and thumb, and, drawing it tense over the latter (which lies below), makes a sudden quick puncture with the lancet. The first drop of blood that exudes should be wiped away with a piece of dry lint, and the next may be used for examination. No pressure must be exerted to cause the drop to appear, otherwise lymph is driven out of its channels, and dilutes the blood, affecting its colour, corpuscle-value, and specific gravity.

(1) **Colour of the blood.** Blood obtained by the method just described appears of a deep red colour, not quite so bright as ordinary arterial blood. The drop may be distinctly paler and more transparent than usual in cases of chlorosis, while it is bright red in carbonic oxide poisoning.

(2) **Reaction.** The blood is an alkaline fluid, due to the presence of di-sodic hydrogen phosphate (Na_2HPO_4) and sodic bicarbonate (NaHCO_3); stated in terms of sodium hydrate, the normal alkalinity is about 0.26-0.3 grms. per 100 c.cm. of blood. It is agreed by competent observers that it is generally diminished in anæmia and chlorosis, and Ewald considers this point as of importance in the etiology of gastric ulcer in chlorotic women. It seems also to be reduced in uræmia, in phosphorus- and carbonic oxide-poisoning, in fevers, in diabetes and in leucocythæmia.

(a) *Qualitative test.* This may be easily performed by allowing a drop of blood to fall on a piece of glazed neutral litmus paper, and washing it off after 10 seconds. A blue spot will remain, indicating that the blood was alkaline. Or a little neutral litmus solution may be allowed to soak into a plate of plaster of Paris and the blood placed upon this. If it is washed off in ten seconds, a blue stain will be found.

(b) *Quantitative estimation.* The carrying out of this is a matter of some difficulty if the titration method (Landois), which is the most accurate, be employed. The easiest clinical process is that devised by Haycraft, which consists in the use of a set of litmus papers containing varying quantities of oxalic acid. That paper which just gives the alkaline reaction in a given time is taken as a measure of the alkalinity of the blood.

R. Hutchison¹ has advanced various objections against this method, the chief of which are that by it the alkalinity of the serum alone is obtained, not that of the corpuscles, and that the more watery the blood, the more readily does it percolate (and therefore affect) the test paper.

Wright² advocates the use of the serum that exudes from ordinary blood-clot, for he holds that changes in the alkalinity of the circulating blood invariably manifest themselves in changes in the alkalinity of the serum. He draws up the serum into a capillary pipette and after it an equal volume of a standard acid solution, *e.g.* 20-, 30-, 40-, or 50-fold dilution of sulphuric acid. The two fluids are then blown out of the pipette into a watch-glass, thoroughly mixed, and the reaction taken. A note is

¹ *Lancet*, vol. i., 1896, p. 615. ² *Lancet*, vol. ii., 1897, p. 719.

taken of the degree of dilution of acid that is just neutralized by the serum, whose alkalinity is then expressed $\frac{35}{N}$, $\frac{40}{N}$, etc., as the case may be. v. Jaksch has also devised a delicate method in which solutions of varying degrees of acidity are employed.

(3) **Specific gravity.** This in healthy men varies from 1057-1066, the average being 1060, while in women it is somewhat lower, ranging from 1054-1061, with an average of 1057. It is normally higher at birth, and the blood may also be concentrated (temporarily) in acute diarrhoea, severe vomiting, and during deprivation of fluids.

The density is lowered by hunger, pregnancy, severe anæmia, and temporarily from transfusion or ingestion of large quantities of fluid. The chief point in this relation is, that the specific gravity varies essentially with the amount of hæmoglobin, and therefore may be taken as a measure of the latter except when there is dropsy. The following table compiled by Hammerschlag gives the ratio between the two:

Specific gravity.		Hæmoglobin Per Cent.		Specific gravity.		Hæmoglobin. Per Cent.
1033-35	=	25-30		1048-50	=	55-65
1035-38	=	30-35		1050-53	=	65-70
1038-40	=	35-40		1053-55	=	70-75
1040-45	=	40-45		1055-57	=	75-85
1045-48	=	45-55		1057-60	=	85-95

Determination of specific gravity. There are various clinical methods, but one of the neatest and simplest is that devised by Hammerschlag.¹ It is based on the fact that blood will not mix with either chloroform or benzol, and

¹ *Zeitschr. f. klin. Med.*, No. xxv., s. 444, 1892.

advantage may be taken of this to make a mixture of these liquids in which a drop may be suspended. The benefit of using chloroform and benzol for this purpose is that they differ widely in their specific gravity.

To carry out the process, a cylindrical urine-glass is thoroughly cleaned and dried, and then rinsed out with a little of the chloroform-benzol mixture. The latter is simply made by mixing together two or three ounces of each of the liquids, so as to have a density of 1050 to begin with; this mixture may be kept in a stoppered bottle. When ready, fill the urine-glass three-fourths full with the mixture; then take a drop of blood from the ear and draw up into a perfectly clean dry pipette (*e.g.* that used for leucocyte counting in the Thoma-Zeiss apparatus), carefully avoiding the inclusion of air-bubbles. Blow out a small portion of the column of blood, wipe it off, and then blow out nearly all that remains till it falls as a drop into the glass. The blood may fall to the bottom of the glass, in which case the mixture is less dense than the drop, and must have its specific gravity raised by the addition of a few drops of chloroform; or it may remain at the top, indicating that it is lighter than the mixture, whose density must then be lowered by the addition of a little benzol. After every addition of these liquids, the whole mixture must be well stirred with a glass rod. At last a point is reached when the blood drop neither rises nor falls, but remains suspended like a red bead in the body of the liquid. It then obviously possesses the same density as the mixture, and on ascertaining this with a clean, dry sensitive urinometer we obtain the specific gravity of the blood.

Comparative examinations should always be made at the same hour of the day. The pipette, glass, etc., must be thoroughly clean and free from water or other foreign element, else the drop may adhere to the side of the glass and the observation be rendered futile. The mixture after use may be filtered through ordinary white filter-paper and used again. Methylated chloroform and purified benzol (80 per cent.) do very well according to the author's experience.

In Schmaltz's¹ process a capillary picnometer, holding 0.1 c.cm. of blood, is used, and the result is obtained by weighing. The author has had no experience of its value.

(4) **Coagulability.** The rate at which blood coagulates varies in different diseases, and can be ascertained clinically by Wright's method. His latest apparatus² consists simply of a set of thick glass capillary tubes, with a calibre of $\frac{1}{100}$ inch in diameter. It is advisable that they be used at a temperature of 18.5° C., and this is secured by slipping a rubber cap over the end of each tube and placing them all in a tumbler of water of that temperature for a few minutes. On removing them, the outside of each is first of all wiped dry, and thereafter the caps are removed. The tubes being thick retain their heat for some little time. They are now filled, to the number of six or eight, with blood from the finger, the time at which each is filled being carefully noted. At varying intervals of time after filling, say 2½, 3, 3½ minutes, and so on, an attempt is made to blow the blood out of the tubes. When the moment is reached at which it becomes just impossible to do this,

¹ Schmaltz, *Deut. med. Woch.*, s. 555, 1891.

² Wright, *Lancet*, Jan. 8, 1898.

the time is noted that has elapsed from the filling of that tube, and this is termed the "coagulation-time." It is usually four minutes in health. The time is greatly extended in hæmophilia and where serous hæmorrhages occur, as in scurvy, urticaria, and chilblains. An increased formation of fibrin is met with in pneumonia and acute articular rheumatism.

Iso-tonic coefficient. When a drop of blood is diluted with distilled water, the red corpuscles become broken up and the hæmoglobin escapes. The addition of certain salts, however, such as sodium chloride, prevents this, and the quantity necessary to keep the corpuscles intact is termed the iso-tonic coefficient. Since 0·44-0·48 per cent. of the salt mentioned (sodium chloride) is the proportion required to keep the cells from destruction in health, the iso-tonic coefficient of normal blood in terms of sodium chloride is said to vary from 0·44-0·48. Under certain morbid conditions this figure appears to be raised or lowered.

Estimation of hæmoglobin. This is performed, in this country at least, chiefly by means of Gower's hæmoglobinometer, though v. Fleischl's hæmometer is also used, but to a much smaller extent. There are several other instruments, such as Hénoque's hæmatoscope, Oliver's hæmoglobinometer, Bizzozero's chromo-cytometer, and so on, but only the two first mentioned will be described in detail.

Gower's hæmoglobinometer. This consists of two cylindrical glass tubes, each about 10·5 cm. high and rather less than 1 cm. in diameter, which can be fixed in a little stand. One of them is filled with a preparation of gela-

tine and carmine of precisely the same colour as a 1 per cent. solution of normal hæmoglobin in distilled water; this is the standard tube. The other is graduated up to 110 in divisions, each equivalent to 20 c.cm. A small pipette for 20 c.cm. is also supplied.

To use the apparatus, first place a drop or two of distilled water in the empty tube; then prick the patient's ear or finger, and draw up by means of the pipette sufficient blood to reach exactly to the 20 c.cm. mark. Gently wipe off any blood on the outside of the pipette, and then blow out the contents into the distilled water in the graduated tube, and mix thoroughly. The pipette should be filled with water once or twice and the latter blown into the tube to ensure that all the blood is removed. It will probably now be found, on comparing this tube with the standard one against a neutral back-ground, *e.g.* a sheet of white paper or a white cloud in the sky, that the former is still the darker. Distilled water must therefore be added cautiously, with constant stirring by the pipette, till the tint of the blood in the measuring tube is exactly the same as that in the standard. If it be found then that the fluid in the graduating tube stand at 100, that is to say, is a 1 per cent. solution, it is clear that the blood possesses its normal amount of hæmoglobin. If, on the other hand, it is so poor in hæmoglobin that when diluted up to, let us say, 45, it gives the same tint as the standard, it obviously contains only 45 per cent. of colouring matter. This method is simple and expeditious; the chief objection to it is that it depends on the exercise of the colour-sense, which varies much in different persons, and which may not be constant even in the same indi-

vidual. The standard tubes supplied vary in different instruments, and when new are frequently too high in colour; they tend to fade after a time. If, however, the observer use the same instrument, and as nearly as possible under the same conditions, it affords a useful clinical method of estimating the amount of hæmoglobin, and especially of recording any alteration in it from time to time.

Observations should always be made at the same time of day, for example, in the forenoon, three hours after a meal.

The hæmoglobin is diminished in all forms of anæmia, and especially in chlorosis, where it is much reduced in proportion to the red cells (see p. 199). After use the graduated tube and pipette must be thoroughly cleansed with water, and the interior of the former should be dried with a little cotton-wool on the end of a probe.

v. **Fleischl's hæmometer** (fig. 25). In this instrument a wedge-shaped piece of glass, tinted red, is made to move underneath a cell filled with distilled water till such a point is reached that the colour of the glass transmitted up through the water has the same tint as blood diluted to a certain degree. From the thickness of the wedge so interposed, the richness of the blood in hæmoglobin can be gauged. The instrument consists of a platform *P*, on which is a small cylindrical glass cell divided into two compartments, *A* and *A*¹, by a vertical partition. Underneath is a reflector *S*, while between it and one of the divisions of the cell is the wedge-shaped piece of glass *K*, clear at one end, and tinted deep red at the other, with uniform gradations between. This

can be moved backwards and forwards by means of a milled head *T*, so that a greater or less thickness is

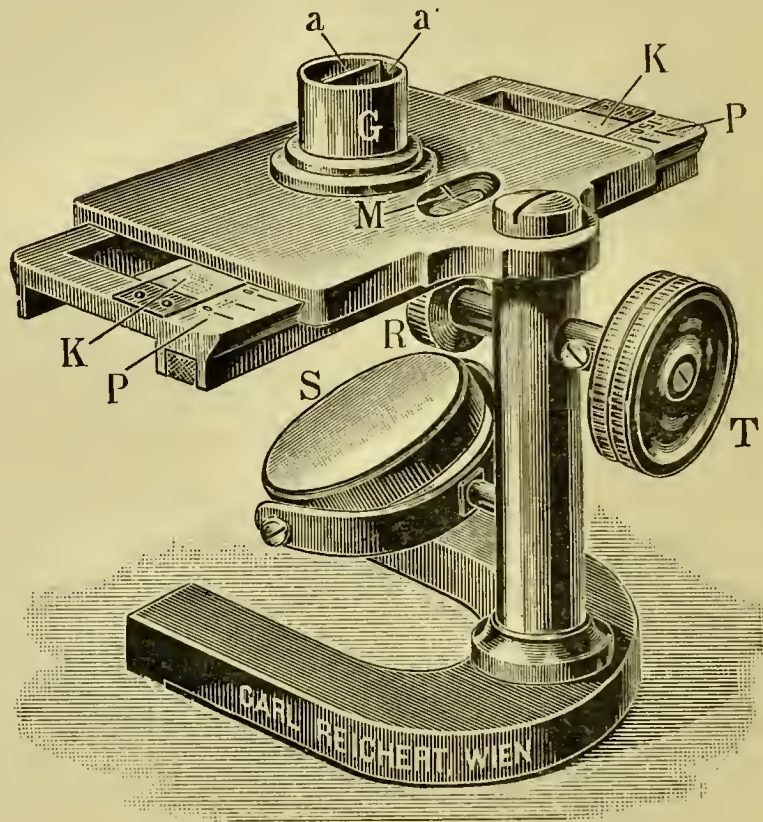


FIG. 25.—FLEISCHL'S HÆMOMETER.

interposed between the reflector and the cell. There is also a small handled capillary pipette for measuring and mixing the blood.

To use the instrument, carry the little cell with the pipette to the bedside, having first placed a little water in one of the chambers of the cell till it is, let us say, one-quarter full. Having obtained a good drop of blood, touch it with the pipette (which must be quite clean and dry), when the latter will fill by capillary action. Having filled it exactly, wipe off any blood that may adhere to the outside, and plunge it at once into the distilled water in the cell. Here it must be freely

moved about by means of its handle, and any blood that will not readily come away may be blown out by means of a piece of pointed glass tubing filled with water. The compartment must now be filled quite to the brim with water (the blood being thoroughly mixed therewith), and the other half of the cell filled with water alone, in the same way. The latter is placed over the wedge of tinted glass, and all is then in readiness to complete the estimation.

This is done in a darkened room by candlelight placed opposite the reflector, while the observer sits so that he looks *along* the partition dividing the cell, not *across* it. As he looks down through the cell, he brings, by means of quick jerky turns of the milled head, a greater or less thickness of the tinted glass to bear on the distilled water till the tint the latter assumes corresponds exactly to that of the diluted blood. All that is necessary then is to read off on a scale the percentage of hæmoglobin the given thickness of glass corresponds to. It is advisable in the use of this instrument to have a paper tube for looking down through, so as to concentrate the light as much as possible. The less light used the better, and the observer should never look for more than a few seconds at a time.

The chief objection to the instrument is the fact that at any given time, since the tinted glass is wedge-shaped, the colour of the water in the cell is not uniform, being darker at one side than at the other, so that we must, as it were, try to get the colour in the middle—not an easy task. Further, it is not easy to estimate the hæmoglobin by this instrument if the percentage is very low. To

obviate this difficulty, Coles¹ recommends that two capillary tubes of blood be used instead of one, and then one-half of the recorded percentage taken as the hæmoglobin value.

Of other instruments, Oliver's hæmoglobinometer is neat, ingenious, and easily worked. It consists essentially of a set of twelve discs of tinted glass, coloured, so as to represent 10-120 per cent. of hæmoglobin. These are used to compare with a solution of the blood under examination. Intermediate degrees (*e.g.* between 10-20 per cent.) are obtained by means of coloured riders, which can be superimposed on the tinted discs. For details, Oliver's² own description of the instrument may be consulted. As already mentioned, the specific gravity of the blood gives a fair measure of its richness in hæmoglobin, and one free from the risk of error contingent on variations in the colour-sense.

Enumeration of red corpuscles. For this purpose the Thoma-Zeiss hæmocytometer is very largely employed; in addition to it, there are Gower's and Oliver's hæmocytometers, and Hedin's and Daland's hæmatocrits.

The Thoma-Zeiss instrument consists of a special slide with a counting area upon it, and a graduated pipette.

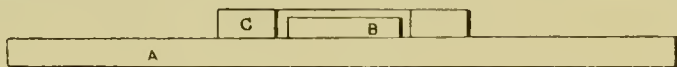


FIG. 26.—THOMA-ZEISS SLIDE. *a*, Slide; *b*, platform; *c*, wall of moat.

The counting chamber consists of a small raised area (fig. 26, *B*) ruled with rectangular lines, which enclose spaces exactly $\frac{1}{400}$ mm. square. Surrounding this area is

¹ Coles, *The Blood and its Diseases*, 1898, p. 15.

² Oliver, *Lancet*, vol. i., 1896, p. 1699.

a sort of trench or moat, and beyond this, again, a little platform exactly $\frac{1}{10}$ mm. higher than the portion with the ruled squares. It is obvious that when a cover-glass rests on the platform it will form a roof for the ruled squares, so that each will, as it were, become a little chamber $\frac{1}{4000}$ c.mm. in capacity. These little squares are arranged in sets of sixteen (four rows of four each), and each set is separated from its neighbour by a triple ruling (fig. 28). Of these sets, there are usually sixteen on a slide.

To use the instrument, see first of all that it is perfectly clean, and especially that no dust or grease rest on the little platform. The pipette also must be clean and dry. It will be observed that the latter is graduated on its stem, and marked at 0.5 and at 1; above this it expands into a mixing chamber containing a glass ball to ensure thorough mixing, and above this there is another line marked 101 (fig. 27). Finally, in order to dilute the blood, it is necessary to have some suitable fluid; for this Gower's solution is frequently employed. Its formula is:

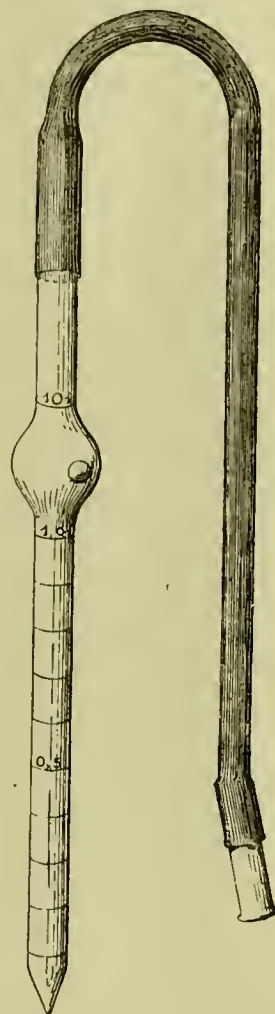


FIG. 27.—THOMA-ZEISS
PIPETTE FOR RED CELLS.

Sodium sulphate,	-	-	-	-	-	104	grs.
Acetic acid, -	-	-	-	-	-	1	drm.
Water, -	-	-	-	-	-	4	ounces.

Or Toison's fluid may be used, which, in addition to preserving the red, stains the nuclei of the white cells slightly. It consists of

Methyl-violet,	-	-	-	-	-	0.025	gram.
Sodium chloride,	-	-	-	-	-	1	gram.
Sodium sulphate,	-	-	-	-	-	8	grms.
Glycerine,	-	-	-	-	-	30	c.cm.
Distilled water,	-	-	-	-	-	160	c.cm.

The patient's ear is pricked in the customary manner, and the tip of the pipette applied to the drop; under capillary action blood enters the tube and is allowed to reach exactly to the line marked 0.5, where we desire a dilution of 1-200, or to that marked 1.0, where a dilution of 1-100 will suffice. If by any mishap the blood is drawn up past the latter point, it enters the mixing chamber, and the trial must be repeated after cleansing and drying the pipette. Having got it exactly at the desired line, the outside of the pipette is quickly wiped free of blood, and the instrument plunged at once into the bottle of diluting fluid, which should be standing at hand uncorked and ready for use. As soon as the point of the pipette is well under the surface of the liquid, aspiration should be performed by the lips, with a simultaneous rotation of the instrument between the finger and thumb, to ensure thorough mixing. As soon as sufficient fluid has been drawn up to reach the point marked 101, the pipette is withdrawn and thoroughly shaken for a minute, its ends being closed by the finger and thumb. We may now place a drop on the counting-slide and complete the enumeration.

Since the fluid, which fills the pipette from the point

to the mixing chamber, consists only of the diluting liquid, it must first be got rid of; this is accomplished by blowing out a few drops, say half-a-dozen, when we may feel sure that the next will consist of diluted blood. The clean counting-slide having been placed on a perfectly level surface, with a cover-glass propped up beside it, a drop of blood of suitable size is blown out upon the little ruled platform. This drop must be large enough to cover at least the greater part of the platform when the cover-glass is laid in position, and yet not so large as to overflow into the surrounding moat. Practice and experience alone enable one to do this properly.

The cover-glass is now let down gently upon the drop, which is flattened out beneath it, and fills up the space between the ruled disc and the cover. If the counting-slide and cover-glass are quite clean, and the latter lying well in position, the observer should be able, by holding the slide almost on a level with the eye, to see concentric coloured rings (Newton's rings) between the glass slip and the slide. Sometimes a little gentle pressure with the point of a needle will make these more apparent.

The slide is now placed on the microscope stage and left there for a minute or two to let the corpuscles settle down. For counting, the ordinary high-power lens (*e.g.* Zeiss D) is employed, and the beginner occasionally experiences some little difficulty in finding the ruled area and the proper focus, but by carefully screwing down, the corpuscles will suddenly come into sight, and the ruled area is found by moving the slide about a

little. Having arranged a set of sixteen small squares in the field (fig. 28), count all the corpuscles in these.

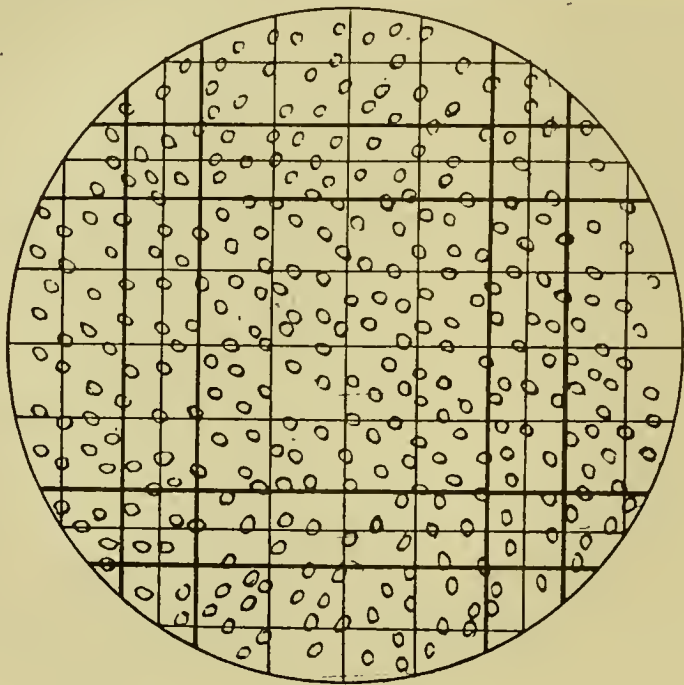


FIG. 28.—COUNTING AREA OF THOMA-ZEISS SLIDE SHOWING RED CORPUSCLES.

Let the eye travel from left to right along each row, beginning at the topmost one. It will be noticed that some corpuscles lie not *in* the squares, but *on* the bounding lines; in such a case count all the corpuscles lying on the lines bounding the left and upper sides of any given square. Having counted the top row in this way, repeat the process with the second, third, and fourth, and then write down the number obtained.

The slide must now be moved so as to bring a new set of sixteen into view, and the corpuscles counted in these, and finally the process should be repeated with a third group. For ordinary clinical work it is sufficient to count three sets of sixteen, but, obviously, the more squares counted, the less will be the degree of error.

Calculation. Suppose that the numbers obtained from three sets (with a dilution of 1-200) were 72, 75, and 69 respectively; this yields a total of 216 for forty-eight small squares, or an average of 4.5 corpuscles to each square. But since each of these has a capacity of $\frac{1}{4000}$ c.mm. and the blood was diluted 200 times, the total number in a cubic millimetre will be:

$$4.5 \times 4000 \times 200 = 3,600,000.$$

The red corpuscles normally number 5,000,000 per c.mm. in the adult man; in women they are slightly below this. Increase in the number is sometimes termed "polycythæmia," and may be met with:

- (a) In the newly-born.
 - (b) In recovery from severe anæmia.
 - (c) In temporary concentration of the blood, as after severe diarrhœa or sweating.
 - (d) In persons living at high altitudes.
 - (e) In persons poisoned by phosphorus or carbonic oxide.
- (Cabot.)

The red cells are diminished (oligocythæmia) in pernicious and secondary anæmias, often to a great degree, but much less so in chlorosis. In the latter it is the hæmoglobin that is notably diminished; it may be reduced, *e.g.* to 30 per cent., while the corpuscles may be 90 per cent. We can thus express the hæmoglobin value of each corpuscle, which, in the example just quoted, would be $\frac{30}{90}$, or one-third of the normal. On every occasion, after the use of the apparatus, the slide and cover-glass must be thoroughly washed and dried before being replaced in their case. The pipette should be

washed out first with water, then with absolute alcohol, and finally with ether; air should then be aspirated, not blown, through it to cause the latter to evaporate.

Oliver's hæmocytometer. The measurements here are based on the degree of dilution the blood must undergo in order that the illumination of a candle at a given distance may just become visible when viewed through a layer of this diluted blood in a flattened glass tube. The poorer the blood is in corpuscles the less dilution will be required, and in this way a table of comparisons can be worked out, on the basis of which the tube to hold the blood is graduated. For details the reader may consult Oliver's¹ own paper on the subject.

Gower's hæmocytometer. In this instrument two pipettes are supplied; by means of one 995 c.mm. of diluting fluid are measured into a mixing vessel, and by the other 5 c.mm. of blood added thereto, and the whole well mixed. A drop of this fluid is then placed on the counting slide, and a cover-glass applied, and kept in position by clips. The spaces on the counting surface have a capacity of $\frac{1}{500}$ c.cm., and it is advisable to count ten of them.

Ex. Suppose 390 corpuscles are found in ten squares, this will give an average of 39 in each.

$$39 \times 500 \times 200 \text{ (dilution)} = 3,900,000 \text{ per c.mm.}$$

The hæmatocrit (Hedin's or Daland's). In this instrument advantage is taken of centrifugal force. A graduated capillary tube is filled with blood, placed in the centrifuge, and subjected to its action for two or three minutes.

¹ Oliver, *Lancet*, vol. i., 1896.

The result is that the corpuscles are packed together at the far end of the tube, which may be so graduated that each large division corresponds to 100,000 cells per c.mm. In this way the enumeration is quickly made.

Ex. If the column of corpuscles reach up to the division 45, it will indicate a total of $100,000 \times 45$ or 4,500,000 per c.mm.

The advantages of this instrument are that it can be used quickly and easily, and that it saves the eye-strain which the use of the Thoma-Zeiss apparatus entails. The drawbacks are that it is not accurate if the corpuscles vary much in shape and size, and that it makes a loud noise when in use.

CHAPTER II.

The blood continued—Enumeration of leucocytes—Blood-plates—Reaction of diabetic blood to aniline dyes—Staining reaction of leucocytes and red cells—Preparation, fixing and staining of blood-films—Varieties of leucocytes—Parasites and organisms in blood—Serum-test in typhoid fever. Bibliography.

Enumeration of leucocytes. As these are much fewer in number than the red corpuscles, a pipette of larger bore is used, so that the dilution obtained is 1-20 or 1-10 instead of 1-200 or 1-100 as with the red cells. For a diluting fluid a weak solution of acetic acid in water (0·3 per cent.) may be used, and if desired, this may be coloured with a little methyl-green or methyl-violet to stain the nuclei of the leucocytes. By the use of such a fluid the red cells are rendered invisible, and counting thereby facilitated.

As the calibre of the pipette is large, a drop of blood of fair size must be obtained; if this is drawn up to the mark 0·5 we get a dilution of 1-20, very suitable for ordinary purposes. The general steps in the process are the same as in counting the red cells; the diluting fluid is aspirated up to the line marked 11, but as the bore of the pipette is large, it should be held horizontally when lifted from the patient's ear, lest the blood run

out, and the bottle of diluting fluid should be tilted when the pipette is plunged into it, for the same reason.

In counting, the observer must run over the whole sixteen sets of small squares, but as it is common to find only two or three leucocytes in each set, this takes but a very short time..

Calculation. Suppose 38 leucocytes are counted in the whole set of 16 large, *i.e.* 256 small squares, and that the blood is diluted 1-20. The number in a cubic millimetre will then be $\frac{38}{256} \times 4000 \times 20 = 11875$.

The leucocytes usually number about 7000 per c.mm., or 1-700 red cells. They vary, however, much more than the latter do, even in health. Increase in their number is termed "leucocytosis," the reverse being "leucopenia." Physiological leucocytosis is met with:

- (1) In pregnancy.
- (2) In the newly-born.
- (3) During the digestion of proteid food.
- (4) After violent exercise.

Pathological increase is met with:

- (1) After hæmorrhages.
- (2) In inflammatory and in infective diseases, notably pneumonia, scarlet fever, diphtheria, peritonitis, and abscess formation.
- (3) Typically in leukæmia (may be 400,000 per c.mm., see p. 212).
- (4) In malignant disease, and more marked the greater the cachexia.
- (5) In many other diseases to a greater or less extent. It can be produced artificially by taking salicylate of sodium or nuclein.

The number found under pathological conditions varies greatly. The author has frequently found 10,000—18,000 per c.mm. in cancer, while in pneumonia 20,000 is an ordinary enough figure. A case of infective disease such as pneumonia, showing a sharp attack and no, or slight, leucocytosis, is held by some observers to merit a bad prognosis.

Diminution of the number of leucocytes, or leucopenia, is met with :

(1) In cases of starvation.

(2) During the course of some infective diseases such as typhoid, influenza, and measles.

(3) In pernicious anæmia. (Cabot.)

As regards the number, the author has seen an example of leucopenia in a case of splenic enlargement, where out of a number of examinations he never found more than 2200 leucocytes per c.mm. In pernicious anæmia they range from 1000-5000, while in chlorosis they are little affected.

Blood-plates. These constitute the third corpuscular element in normal blood, and are small colourless or very pale yellow round cells, 2-3 μ in diameter. In health they are present to the extent of 250,000-300,000 per c.mm. They are very adhesive, and readily run together, forming clumps from which little threads of fibrin run out. They may be seen in a fresh preparation if examined promptly, or the blood may be diluted with Hayem's solution (Hydrarg. perchlor. 0.5 grm., sodium sulph. 5.0 grms., sodium chlor. 1.0 grm., aq. destil. 200 c.cm.).

They are increased in leukæmia, and often in per-

nicious anæmia, and seem to be associated with the clotting of the blood.

Reaction of diabetic blood to aniline dyes. Bremer¹ pointed out, a few years ago, that if films of blood from cases of diabetes or glycosuria were spread on cover-glasses, fixed by chloroform and ether, and stained in a special way with an alcoholic solution of a powder obtained from the interaction of eosin and methylene-blue, it would be found that they presented a bluish-green appearance, while healthy blood treated in a similar way looked reddish-violet. Microscopically, the red cells appeared green in diabetic blood, and purple in that in health. This condition is not due to sugar, but, on the other hand, films of healthy blood floated for a few minutes on diabetic urine stain like diabetic blood.

Bremer,² in a later paper, advises that a drop of blood be spread over one-third of a slide to form a film, control slides of healthy blood being made at the same time. The slides are heated for six to ten minutes in an oven at 135° C., and when cool placed in a dish containing a 1 per cent. watery solution of Congo red or methylene-blue for one and a half to two minutes. It will then be found that diabetic blood is not stained at all, or indifferently so, while normal blood stains well. Biebrich scarlet, used in a similar fashion as the stain, acts conversely, staining diabetic, but not non-diabetic blood.

Staining reactions of leucocytes and red blood cells. Modern study of the blood, particularly of the leucocytes, has shown that of the latter class of cells different forms

¹ Bremer, *New York Med. Jour.*, 1896, p. 301.

² Bremer, *Centralbl. f. inn. Med.*, 1897.

have affinities for different stains, and it has become possible, in this way, to produce a differential staining of the blood. In normal blood, the following varieties of leucocytes occur. (Cabot.)

(1) Small lymphocytes, about $10\ \mu$ in diameter, and taking up basic stains, such as methylene-blue, which stain the large nucleus deeply—20-30 per cent.

(2) Large lymphocytes and transitional forms, $13\ \mu$ in diameter. These also stain with basic dyes—4-8 per cent. (Nos. 1 and 2 are young cells.)

(3) Polymorphonuclear cells, or neutrophiles, $13\ \mu$. They are adult cells, and have a twisted nucleus, which takes up acid dyes, while around it are "neutrophile" granules, which colour with neutral stains—60-72 per cent.

(4) Eosinophiles; these are old cells, and measure $12\ \mu$ in diameter. The nucleus stains rather pale, but the granules which stud the protoplasm have a great affinity for acid dyes, such as eosin— $\frac{1}{2}$ -4 per cent.

(5) Basophile "mast-cells," with granules that take up basic stains may also occur— $\frac{1}{4}$ -4 per cent.

The red cells usually stain well with acid stains, such as eosin, acid fuchsin, and aurantia.

The preparation, fixing, and staining of blood films.

(1) *Preparation.* In order to obtain good specimens it is first of all essential that the observer be provided with proper cover-glasses. Those known as "No. 1 squares," either $\frac{3}{4}$ or $\frac{7}{8}$ in. in diameter, are the proper kind to use. They must be thoroughly washed with soap and warm water before use, to remove all dirt, and especially all grease, and then thoroughly dried with an old soft silk handkerchief. When ready they may be propped up

against some suitable object, so as to be at hand when required.

The patient's ear having been cleansed and punctured in the usual way, the observer lifts two cover-glasses by their edges between the thumb and forefinger of the right and left hands, and having just touched the apex of the drop of blood with the centre of one glass, he lets it fall gently on the surface of the other. In doing so it must be arranged that a corner of the upper cover-glass coincide not with a corner but with the middle of a side of the lower one, so that the projecting angle can be seized when required. The blood spreads itself out at once, forming a thin layer between the cover-glasses, which may now be separated by a gentle sliding motion. Each slip will be found to be covered with a tolerably uniform thin film of blood.

Examination of fresh blood. Such a film may be examined at once in the fresh condition, when information will be gained as to the formation of rouleaux by the red cell, the presence of excess of fibrin, the relationship of the number of white to red cells, and the presence of deformity in the latter, or "poikilo-cytosis." Blood may also be conveniently examined by mixing it with a little physiological salt solution (0.75 grm. of sodium chloride to 100 c.cm. of water). All fresh specimens soon undergo changes from exposure to the air, but if the latter be excluded as far as possible, the onset of these alterations is delayed. This is best accomplished by painting on the slide a hollow square of vaseline of such size that, when the cover-glass is lowered upon it, its edges are embedded in the vaseline all round.

Fresh blood may be fixed at once by placing a drop of 2 per cent. osmic acid solution on the ear, and pricking through it. The red cells can then be well studied.

(2) *Fixation of films.* This may be done in one of two ways, either by the use of chemical agents or by heat. In the former case the simplest procedure is to drop the freshly-made films at once into a mixture of alcohol and ether in equal parts, kept in a wide-mouthed, stoppered bottle. It is generally advised that they stay there for from fifteen to thirty minutes, but Coles¹ states that three to five minutes' immersion is sufficient for staining with eosin and hæmatoxylin. If the films are left longer than half an hour no harm is done.

In Gulland's method (see below), where staining and fixing are accomplished at the same time, a mixture of ether, alcohol, and corrosive sublimate is used.

Formol (40 per cent. aqueous solution of formic aldehyde) is also a good fixative; a suitable mixture is formol one part, water nine parts, and alcohol ninety parts. An immersion of one minute suffices.

When fixing is done by heat, the films should be placed in a hot chamber at a temperature of 115° C. for fifteen minutes. A very fair substitute for such a chamber may be improvised in this way. A flat bar of copper 38 cm. (15 inches) long and 5 cm. (2 inches) broad is supported on a ring of the retort stand, and heated below by a bunsen burner. In a short time such a bar will have a fixed temperature at any given point, and in order to find the point where it is 100° C., all that is requisite is to place a series of drops of water along the bar, and note

¹ Coles, *Op. cit.*, p. 25.

the point at which a drop just boils. At this spot the cover-glasses should be laid film side down, and left so for from fifteen to thirty minutes.

In a case of great haste the film may be fixed rapidly by holding the cover-glass between the finger and thumb over a bunsen flame, and as near as the skin can bear, for a space of forty seconds.

(3) *Staining of the films.* (a) *Gulland's*¹ *method.* In this beautiful and simple process, staining and fixing are carried out simultaneously. The films having been made in the usual way, the cover-glasses are at once laid, wet side downwards, in a little (5-10 c.cm.) of the solution in a small flat dish. The solution is composed of:

Absolute alcohol saturated with eosin, -	-	25 c.cm.
Pure ether, - - - - -	-	25 c.cm.
Corrosive sublimate in pure alcohol, -	-	5 drops.
(2 grms. to 10 c.cm.),	(more or less.)	

To be kept in a wide-mouthed well-stoppered bottle.

In cases where it is desired to make films at the bedside and examine afterwards, the cover-glasses may be dropped at once into the bottle. The films should remain in the fluid for five minutes at least, but it is immaterial if they stay there for a whole day; after being left there for the requisite time they are lifted out by a pair of forceps, waved to and fro in a small basin of water to wash thoroughly, and then stained for exactly one minute in a saturated watery solution of methylene-blue; thereafter they are again washed quickly in water, dehydrated in absolute alcohol, cleared in xylol, and mounted in xylol balsam.

¹Gulland, *Brit. Med. Jour.*, vol. i., 1897, p. 652.

The red corpuscles are stained pink, and nuclei of cells a deep blue; the bodies of the leucocytes are in varying shades of pink, while the eosinophile and basophile granules are well brought out.¹

(b) *Ehrlich's triple stain.* This is made up as follows:

Biondi-Heidenhain powder, ²	-	-	-	-	grs. xv.
Absolute alcohol,	-	-	-	-	1 c.cm.
Water,	-	-	-	-	6 c.cm.

(Mix.)

A little of this mixture is poured into a watch-glass and the film, already fixed, is floated on it for from one to five minutes. If it has been fixed by heating on the copper bar (see above) for, say, fifteen minutes, it is best to stain for four or five. After removal from the stain the cover-glass is washed well in water, dried between two pieces of filter-paper, and mounted in Canada balsam. With Ehrlich's triple stain the red corpuscles stain a bright yellow; the small lymphocytes have the protoplasm pale pink and the nucleus blue; the large lymphocytes and transitional forms show a rather pale staining of both nucleus and protoplasm; the eosinophile cells have a pale nucleus, while the eosinophile granules show large and distinct and of a copper or burnt sienna colour; and lastly, in the polynuclear neutrophiles, the twisted nucleus is stained blue or greenish blue, and the small granules purple, violet, or pink.

(c) *Staining with eosin and hæmatoxylin.* This is warmly

¹ Further details of this and other methods will be found in a paper by Gulland in the *Scottish Medical and Surgical Journal*, April, 1899.

² The constituents of the powder are Orange G., acid fuchsin, and methyl-green, and it can be obtained best from Grübler and Co., Bayer'sche Strasse, Leipzig.

advocated by Coles as a good and reliable method. Films are made on a slide by drawing the edge of a second slide, held at right angles to the first and inclined at an angle of 45°, over a drop of blood. The films, dried in the air, are fixed for a minute or two in an alcohol-ether bath. They are then stained for half a minute or longer in the following :

Eosin, -	-	-	-	-	-	-	-	1 part.
Alcohol,	-	-	-	-	-	-	-	100 parts.
Water,	-	-	-	-	-	-	-	100 parts.

Wash then in water and stain for from ten to fifteen seconds in Delafield's hæmatoxylin solution, which should be filtered before use. Wash again in water and examine wet to see if the nuclei of the leucocytes are of a sufficiently deep blue ; if not, stain a little longer with hæmatoxylin. Wash finally in faintly alkaline water, dry, clear with xylol, and mount in balsam. The red corpuscles appear of a yellow pink or red colour, according to the time they have been stained by the eosin. The nuclei of nucleated red cells show very distinctly. The nuclei of the polymorphonuclear leucocytes appear of a dark blue colour, the protoplasm of leucocytes pink, while the eosinophile granules are stained deep red.

In studying the minute structure of blood cells a $\frac{1}{12}$ in. oil-immersion lens is a great advantage, but much can be made out with an $\frac{1}{8}$ in. objective.

In addition to the varieties of leucocytes which occur in healthy blood, it is necessary to mention the cells called "myelocytes," which appear to arise from bone-marrow, and which are found in the blood under various pathological conditions, notably in spleno-myelogenous

leukæmia and severe anæmias. They are large cells, 15-16 μ in diameter, and when stained by Ehrlich's method appear as spherical bodies with a large round or oval pale-stained nucleus surrounded by neutrophile granules. In splenic leukæmia these cells may compose 30, 40, or 50 per cent. of the total leucocytes, which are themselves often enormously increased. The eosinophile cells are also more plentiful than in normal blood.

In lymphatic leukæmia the leucocytes are not quite so numerous as in the form just described, but the lymphocytes are greatly increased, and may form 90 per cent. of the white cells present.

Abnormal red corpuscles. One may find aberrant varieties of red cells in blood films, especially in cases of pernicious anæmia. They assume three forms :

(1) Normoblasts—of the size and colour of an ordinary red cell, but provided with a round nucleus in size equal to half the diameter of the cell, and placed eccentrically. The nucleus shows deep blue with Ehrlich's stain, the rest of the cell being light yellow.

(2) Megaloblasts—very large cells, not quite round, and occupied in great part by a large nucleus. The latter stains green with the tri-colour stain, and the protoplasm brown.

(3) Microblasts—cells similar to normoblasts as regards the nucleus, but with less protoplasm round the latter.

Eosinophilia. This term is applied to an increase in the percentage of the eosinophile cells, whether the total number of leucocytes is increased or not. It is found, according to Neusser,

(1) In sarcoma, osteomalacia, and other bone affections.

(2) In pemphigus, urticaria, psoriasis, and other skin diseases.

(3) In diseases of the female genital tract, *e.g.* ovarian tumours and gonorrhœa, and in puerperal mania.

(4) In neurasthenia, hysteria, psychoses of menstruation, Graves' disease, and other disturbances of the sympathetic nervous system.

Parasites in the blood. In a work of this size it is impossible to describe more than two or three of the commoner parasites that occur in the blood. More extensive information must be sought for in larger text-books, especially as regards the study of pathogenic organisms. Of the microbes of interest that have been described recently may be mentioned the *bacillus pestis*, the cause of bubonic plague, which occurs in the blood and internal organs, and the *bacillus icteroides* of yellow fever (Sanarelli), which is found in the blood and the tissues. Cases of anthrax may show the *bacillus anthracis* in the blood, but these may not occur in considerable numbers till shortly before death. In cases of septicæmia, pyæmia, malignant endocarditis, etc., bacteriological cultures of the blood may yield important information. The organism of Malta fever (*micrococcus melitensis*, Bruce) has not yet been isolated from the blood.

(1) *Filaria sanguinis hominis*. Of this parasite there are several varieties. The two most important are the *Filaria Bancrofti* (nocturna) and the *Filaria diurna*. The embryos of the former may often be found in the blood of patients whom it has infected, and can be recognized under a low power of the microscope. The embryo is a worm-like body, $\frac{1}{70}$ to $\frac{1}{90}$ in. in length, and a little broader

than the red blood cells, among which it may be seen moving about. In examining the blood, it is well to dilute it with a little normal salt solution, to which some stain, such as methyl-violet, may be added. Or a film of blood may be dried on a slide, stained for thirty seconds with a 2 per cent. aqueous solution of methylene-blue, washed well in a very dilute solution of acetic acid in water, allowed to dry, and mounted with Canada balsam. Other methods of demonstrating the parasite will be found in detail in Manson's¹ excellent work on tropical diseases. The embryos are only found in the blood during the night, or when the patient is asleep.

In the other variety, *F. diurna*, the embryo is much the same as that already described, but it is found only by day or when the patient is awake. A third variety, *F. perstans*, has recently been described by Manson,² who has found it in the blood in many cases of the peculiar African disease called "Sleeping-sickness." He found it, however, also in apparently healthy persons, but limited to the region of the Congo, in West Africa. It is $\frac{1}{125}$ in. long and $\frac{1}{5000}$ in. broad, has a blunt tail and no sheath, moves actively through the blood, and is present in the peripheral circulation both by night and by day. It has quite recently³ been shown to be identical with the immature form of a nematode worm, endemic in tropical America.

(2) *Spirillum* or *Spirochæte* of relapsing fever. Blood diluted with normal saline solution and examined during

¹ *Tropical Diseases*, Dr. Patrick Manson, 1898.

² Manson, *Brit. Med. Jour.*, vol. ii., 1898, p. 1676.

³ *Brit. Med. Jour.*, vol. i., 1899, p. 429.

a paroxysm of the fever may reveal the presence of this organism, which is in the form of a slender spiral, whose length is equal to from three to six times the diameter of a red blood cell. They are absent during the periods of intermission.

(3) *The parasites of malaria.* Of great importance is the examination of the blood for the parasite of malarial fever. This subject has aroused much interest of late years, especially with reference to the part played by insects, *e.g.* the mosquito, in transmitting the disease from man to man, or from lower animals to man. The parasites are hæmatozoa, that is to say, they live and pass through various cycles of their life history within the red corpuscles. Not only can the diagnosis of the disease be made from the discovery of the parasites in the blood, but the variety of the fever (tertian, quartan, etc.) can be determined according to the size, kind of pigmentation, length of life-cycle, etc., of the hæmatozoon found.

Life-history of the parasite within the body. To condense a description given by Manson, if one examine the blood of a patient just before the stage of rigor, inside a certain number of the red cells, a disc of protoplasm will be found dotted over with black specks of pigment. If the blood be examined during rigor, the pigment particles are found more or less concentrated in the centre of the corpuscle, and the surrounding pale protoplasm is broken up into a series of spherules. Next, if

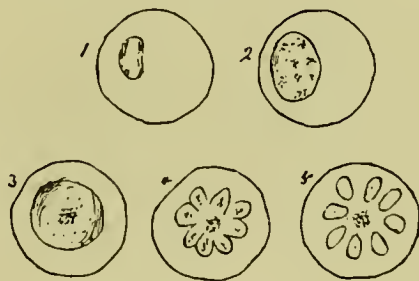


FIG. 29.—DIAGRAMMATIC SCHEME OF LIFE-CYCLE OF MALARIAL PARASITE.

the blood be examined during the stage of pyrexia, it will be observed that the spherules have escaped from the corpuscles into the liquor sanguinis, and that some of them have entered fresh red blood cells. Finally, during the stage of apyrexia, one finds these small hyaline intracorpuseular spherules increase in size, acquire amœboid movement, become pigmented, and by degrees arrive at the stage at which this description began (fig. 29).

Sporulation is always attended by a paroxysm of the fever; according as the cycle described above is completed in 24, 48 or 72 hours we deal with quotidian, tertian, or quartan ague. As already mentioned, there are differences between the parasites of the various forms of malaria, and these are indicated in the table on p. 218. Mannaberg¹ gives the following simple classification of the parasite:

I. Malarial parasites with spore-formation without crescents.

(a) Quartan parasite.

(b) Tertian parasite.

II. Malarial parasites with spore-formation, and with crescents.

(a) Pigmented quotidian.

(b) Unpigmented quotidian.

(c) Malignant tertian.

In the latter group are included æstivo-autumnal, irregular, and malignant forms of malaria.

The bodies termed *crescents*, or *Laveran's corpuscles*, are formed inside red corpuscles. They are of the shape of the new moon, measuring 8-10 μ from horn to horn, and

¹ "The Malarial Parasite," *New Syden. Soc.*, 1894, p. 332.

they always contain pigment. They may become round or oval, and are then termed *spherical bodies*.

Flagellated bodies consist of a central sphere dotted with pigment, and proceeding from it 2-6 actively motile filaments or flagella. These elements are only formed, according to Manson, after the malarial parasite has left the body. After a time the flagella break off from the central body and move about freely. Manson¹ regards their formation as a phase of the extra-corporeal life-history of the parasite, and Ross has shown that certain species of mosquitos fed on malaria-affected persons exhibit in their bodies the formation of flagella, and the separation of these from the central round body.

Examination of the blood for malarial parasites. The best time to do this is towards the end of an apyrexial period, just before the paroxysm begins. The cover-glasses, slides, etc., must be thoroughly clean and absolutely dry. The patient's ear must be washed with soap and water, then with alcohol, and dried completely. Make a slight prick, and touch the apex of the exuding drop with the centre of a cover-glass, letting it fall at once upon a slide to form a very thin film. Make half a dozen films at a time, and surround each with a ring of vaseline to exclude the air. In this way we may examine the blood (1) *fresh*.

To examine, use a $\frac{1}{12}$ in. oil-immersion lens; it is absolutely necessary that the corpuscles in the field lie on the flat, not on their edges.

(2) *Films dry but unstained.* Blood films may be spread

¹ "The Mosquito and the Malaria Parasite," *Brit. Med. Jour.*, vol. ii., 1898, p. 849.

TABULAR CHART OF THE CHARACTERISTIC

	Duration of Disease.	Movement.	Pigmentation.	
Simple Intermittent Fever.	QUARTAN PARASITE.	72 hours.	Small movement when immature.	Coarse granules—little or no movement.
	ORDINARY TERTIAN PARASITE.	48 hours or less.	Active movement (amœboid) when immature and middle-aged.	Fine granules in immature forms, often swarming in those larger.
Malignant or Estivo-Autumnal Fever.	PIGMENTED QUOTIDIAN PARASITE.	24 hours.	Actively amœboid in immature unpigmented forms, less so in pigmented.	Very fine, later coalescing into one or two lumps; does not swarm.
	UNPIGMENTED QUOTIDIAN PARASITE.	24 hours or less.	Very active amœboid movement.	None.
	MALIGNANT TERTIAN PARASITE.	48 hours.	Active, and remains in the pigmented bodies.	Moderately fine, may show oscillatory movements.

SIGNS OF THE VARIOUS PARASITES.

Maximum Size.	Number of Spores.	Creseent Bodies.	Form of Spore Formation.	Changes in the Infested Red Blood Cells.
Size of red blood cell.	6-12.	None.	Daisy form, each spore round, with nucleolus.	Little discoloured and not altered in size.
Size of red blood cell, sometimes larger.	15-20, often less.	None.	Sunflower-like, single spores round and small.	Often hypertrophied—colour completely lost.
$\frac{1}{4}$ - $\frac{1}{3}$ size of red blood cell.	6-8, even more.	Present.	Irregular heaps.	Often shrunk and either dark-stained or completely decolorized.
$\frac{1}{6}$ - $\frac{1}{4}$ size of red blood cell.	6-8.	Present.	Star-shaped or irregular heaps.	Frequently shrunk and darkly stained.
$\frac{1}{8}$ - $\frac{2}{3}$ size of red blood cell.	10-12, rarely 15-16.	Present.	Irregular heaps.	Frequently shrunk, dark-stained, or colourless.

on a slide, dried by means of heat, covered and sealed in by a ring of Canada balsam.

(3) *Films dried and stained.* Films may be used on slides or cover-glasses, fixed for five minutes in alcohol and ether, and then stained for 30 seconds in

Borax,	-	-	-	-	-	5 grms.
Methylene-blue,	-	-	-	-	-	0·5 gm.
Water,	-	-	-	-	-	100 c.cm. (Manson).
Dry, clear with xylol, and mount in balsam.						

Coles¹ says that Thionin-blue (1 per cent. solution in 1-40 carbolic acid solution) is a good single stain for malarial films. For double staining, he advocates the use of eosin and hæmatoxylin, which should be employed as follows :

Fix the films in alcohol and ether for three to five minutes. Stain for half a minute, more or less, in a 0·5 per cent. solution of eosin in 50 per cent. alcohol. Wash and stain while wet for half a minute or more in Delafield's matured hæmatoxylin (first filtered).

Wash in filtered water, dry in the air, clear with xylol, and mount in Canada balsam.

The table on pages 218, 219, condensed from Mannaberg's,² indicates the leading characteristics of the different parasites.

Widal's serum test in typhoid fever. This test depends upon the fact that the serum of blood taken from patients suffering from typhoid has a peculiar effect on active motile typhoid bacilli in a fresh culture, causing them to slow in their movements, to adhere together, and finally to form agglutinated masses. The reaction is usually

¹ Coles, *Op. cit.*, p. 193.

² Mannaberg, *Op. cit.*, p. 372.

seen about the seventh day of the disease, and may continue to be shown by the patient for many months. It is held by many to be of considerable diagnostic value, but it is by no means infallible.

If carrying it out, it is necessary to have at hand a fresh culture of typhoid bacilli in bouillon, not more than forty-eight hours' old at the outside. The patient's skin is cleansed and pricked, and a drop of blood sucked up into a small glass bulb; if it coagulate here it does not matter, as a drop of the serum can be blown out for use. A small cover-glass is then taken, and on it are placed by means of a small platinum loop nine drops of the bouillon containing the active typhoid organisms. One drop of blood-serum of the same size is added, and the whole mixed and then examined with the high power. If the reaction be positive, the actively moving organisms begin to travel more slowly, then to adhere, and finally to form motionless masses or clumps. This last stage may be reached in periods varying from one or two minutes to half-an-hour.

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SECTION V.

THE FÆCES.

Quantity—Form and consistency—Odour and colour—Reaction—Naked eye and microscopical appearances—Intestinal parasites—Protozoa—Cestoidea—Trematoda—Nematoda—Chemical examination—Blood—Bile—Stercobilin—Cholesterin—Mucin—Albumin.

BEFORE considering the application of any chemical or microscopical methods to the examination of the fæces, it is necessary that attention be paid to various general points.

(1) **Frequency of evacuation.** This varies considerably, even in health, and in each case it is necessary to find out what is the normal for that individual. Among healthy men a regular morning evacuation is an ordinary state of affairs, but women will often have a movement only once in two or three days, and yet appear to enjoy excellent health.

If a person go distinctly beyond the period when the bowels usually move, they become *constipated*, which is a relative term, while, if the motions are distinctly more frequent than usual, and particularly if they are watery

and associated with pain, the term *diarrhœa* is used. Infants usually have two or three movements of the bowels in twenty-four hours.

Constipation exists in obstruction of the bowel, whatever be its cause, in many cases of fever, in meningitis, in cases of atony of the intestinal wall, in acute and chronic peritonitis, in dilatation of the stomach, and in certain forms of spinal cord disease.

Diarrhœa occurs typically in cholera, enteric fever, and dysentery; also in acute and chronic catarrh of the stomach, in tubercular and waxy disease of the bowels, in cirrhosis of the liver, and after the ingestion of irritating and indigestible articles of diet.

(2) **Quantity.** This varies in health from 100 200 grms. ($3\frac{1}{2}$ -7 oz.) per diem, but depends both on the size and number of the meals and on the nature of the food.

(3) **Consistency and shape.** (a) The consistency of normal fæces is well known; it may alter in the one direction in the case of constipation when the motions may be dry and hard (if rounded and ball-like they are termed *scybalæ*), or, in the other direction, in diarrhœa, where they may have the consistency of a thick, thin, or watery fluid.

(b) The shape is normally cylindroid; in cases of narrowing of the rectum from side to side, *e.g.* by the pressure of a tumour, the shape of the motion is altered in conformity, and becomes flattened or ribbon-like, and in some cases grooved.

(4) **Colour and odour.** (a) The colour of the healthy stool in the adult is dark brown; in infants it is a light yellow. In disease it varies very much, and may also be

altered during the administration of drugs. The nature of the food may likewise affect the colour, which is notably paler during a milk diet.

When blood is present (*melæna*) the motions are usually dark, even black, generally offensive, and frequently termed 'tarry.' If the source of bleeding be near the rectum, the blood may stain the *fæces* red.

In obstruction of the bile-duct (*acholia*) the motions are pale, sometimes white, and are suitably described as 'clayey.' In some cases unaltered bile-pigment may colour the *fæces* a greenish-brown; this may result from an abundant biliary outflow and concomitant rapid peristalsis, whereby the pigment has not time to become converted into the *fæcal* pigment *stercobilin*.

Diarrhœic stools are usually paler than normal, and in cholera are quite colourless or slightly opalescent, 'rice-water' stools. In young children the stools may be green, due in part to the presence of bile, and in part probably to the action of certain micro-organisms which develop a green colour. As regards drugs, iron, bismuth, and manganese impart a black colour to the *fæces* from the formation of their respective sulphides; charcoal also imparts a black tint. Calomel often makes the motions green, while senna and rhubarb may give them a yellow hue.

(*b*) The odour of the *fæces* in health does not require description; it is due to the presence of indol, skatol, fatty and oily acids, and other products of bacterial action. As already mentioned, the odour of motions containing altered blood is very offensive, and the clayey stools in *acholia* may also be markedly disagreeable. The

motions of children affected with gastro-intestinal catarrh are often sour and unpleasant to smell.

(5) **Reaction.** This is not constant even in health, but as a rule is feebly acid. It may be more markedly so in some cases of acute catarrh of the bowel, and in typhoid may be distinctly alkaline.

Naked-eye appearances. Much may be learned from this in addition to the alterations in colour, etc., which have already been described. Mucus may be found in a greater or less degree, and occasionally complete or partial casts of a portion of the canal in cases of enteritis. Where there are bleeding piles, it may be observed that the motions are, as it were, sprinkled with florid blood. Undigested food and foreign bodies are not uncommonly met with, notably masses of curdled milk, undigested fat, kernels and portions of the shells and skins of fruit; also pus, gall-stones, coproliths or concretions formed in the bowels, and parasites such as thread-worms, round worms, the head and segments of tape-worms, etc. The discovery of gall-stones in fæces is often an important point in diagnosis, and in cases of biliary colic careful search should be made for them. To do this, let the motions be mixed thoroughly with a weak solution of salt and water, and then passed through a sieve. If stones are found, they may be either more or less smooth and round, or they may be facettèd. In the former case there may have been only one stone formed; in the latter they are multiple, the facets indicating the points where the stone was joined to its neighbours.

Microscopical examination. This is often of value clinically, and can be best carried out by mixing a particle

of fæces on a slide with a little normal saline solution, and then applying a cover-glass. If the motions are very liquid no dilution is required. The principal objects which occur are :

(1) Fragments of undigested food, notably vegetable cells, starch granules, striped muscle-fibre, and strands of connective tissue.

(2) Epithelial cells, usually squamous, but sometimes columnar cells which have become altered and shrunken.

(3) Blood corpuscles, usually the red, rarely leucocytes ; pus-cells, however, may occur abundantly under special conditions.

(4) Crystals, such as those of ammonio-magnesium and calcium phosphate, calcium oxalate, fatty acids (arranged in needles), cholesterin and hæmatoidin, and Charcot-Leyden crystals.

(5) General débris and enormous numbers of bacteria.

(6) The ova of parasites, especially those of oxyuris vermicularis and ascaris lumbricoides.

The study of the various pathogenic and non-pathogenic microbes that occur in the fæces falls entirely within the domain of bacteriological work. We need merely mention here that the most important germs found in the motions are :

(1) The coma-bacillus of Koch in cholera asiatica.

(2) The typhoid bacillus of Eberth.

(3) The Finkler-Prior bacillus of cholera nostras.

(4) The bacillus coli communis.

(5) The tubercle bacillus.

Intestinal parasites. These are frequently met with, and are of much practical importance, not only on account

of the local distress they may occasion, but also because of the reflex symptoms they may evoke in distant parts, especially in the case of children.

I. **Protozoa.** Various members of the rhizopoda and infusoria may occur in the fæces; the most important of these is a member of the first-named group, the *amœba coli*, described by Lösch (*amœba dysenteriae*, Councilmann). It occurs as a contractile, more or less round cellular body, three to four times the diameter of a red blood cell. It is specially associated with dysentery, enteritis, and hepatic abscess.

II. **Vermes.** These include two groups, the flat and the round worms, both occurring as parasites in man.

(1) **Cestoidea.** To this order belong the tape-worms, a well-known and common group of intestinal parasites.

(a) *Tænia solium*. It is often stated that this is the commonest variety of tape-worm met with in this country, but it is doubtful if the variety to be described next (*T. mediocanellata*) does not occur more often. The adult worm may attain a length varying from 2 to 4 metres. It possesses a small head about the size of that of a pin, a slender neck, and a body made up of many sexually mature segments called proglottides, which are shed from time to time. These segments are filled with eggs, and may be eaten by pigs, in whose tissues the eggs mature till they reach the larval form called a cysticercus.

The head of *T. solium* is provided with four suckers and a double row of hooklets. One can never be quite certain that a patient is cured of tapeworm till the head has been found in the stools. When one is searching for it, the motions should be well mixed with water,

allowed to settle, and the watery layer poured off; this is repeated several times, and then the sediment is searched for the little round head.

The proglottides are 1 cm. (about $\frac{2}{5}$ in.) long, and 6 to 7 mm. broad. They are largely occupied by a coarsely but not extensively branched uterus, which has a thick central stem (fig. 30). At the side of the segment is



FIG. 30. — SEGMENTS OF *T. SOLIUM* SHOWING COARSELY-BRANCHED UTERUS. $\times 3\frac{1}{2}$. (Leuckart.)



FIG. 31.—SEGMENT OF *T. SAGINATA* SHOWING MUCH-BRANCHED UTERUS. $\times 2$. (Leuckart.)

placed a cloacal orifice, into which the vagina and sperm-duct lead. The segments can be studied to advantage by pressing one flat between two glass plates, *e.g.* two microscope slides. Their structure can be seen to greater advantage if they are first dehydrated with absolute alcohol and then examined in a little oil of turpentine, which clarifies them.

The ova are oval, provided with a thick shell, and exhibit slight radial striation; when mature they contain a small embryo with six hooklets.

(b) *Tænia mediocanellata* (vel *saginata*). This is a larger worm than that just described, and may attain a length of 5 to 8 metres. The other important points of difference between them are as follows :

The head is twice or thrice as large as that of *T. solium*, and has suckers, but no hooklets, while the proglottides are half as long again (15-18 mm.), and average 8 mm. in breadth; they are filled with a uterus that is much more finely branched than in the *solium*, and the branching is more uniform (dichotomous) (fig. 31). Lastly, the cysticercus stage is usually passed in cattle, not in pigs. The ova of these two species are very like one another.

(c) *T. echinococcus*. In this species it is not the adult worm that is of great importance, but the cysticercus which, developing in internal organs such as the liver, gives origin to the 'hydatid' cyst. The *Tænia* itself is very small, 4-5 mm. long, and composed of three or four segments only. The last or mature segment may contain a large number of eggs. The adult worm may occur in the dog. The eggs are ruptured by digestion and the embryo, provided with six hooks, passes through the walls of the intestine, and often finds its way to the liver. Losing its hooks there, it begins to develop into a little cyst, with an external laminated layer (ectocyst), and an inner granular one (endocyst), filled with clear fluid. As the cyst grows larger, daughter cysts bud out from the granular endocyst, and may, as they increase in size,

come to lie free in the mother cyst. From the granular lining there eventually arise 'scolices,' provided with a circle of hooklets and four suckers, and these, if they reach a suitable medium, develop into the adult worm again.

For diagnostic purposes, the contents of the larger cysts may be aspirated off; the clear fluid often contains hook-



FIG. 32.—*a*, HYDATID ECTOCYST; *b*, HOOKLETS; *c*, HEAD OF SCOLEX.

lets, portions of scolices, or fragments of the laminated ectocyst (fig. 32). The fluid (see p. 237) is of low specific gravity (1009), and contains little albumin, but often a trace of sugar. Hydatid cysts may sometimes rupture spontaneously.

(*d*) *T. cucumerina*. This variety occasionally occurs in man, but is more common in the dog. It is not so long as the two first described.

(*e*) *Bothriocephalus latus*. This is a rare cestode in this country, but occurs in Russia, Switzerland, and elsewhere. It is a long worm, and the larva develops in fish. In the case of all these worms the diagnosis is made certain by the discovery of the segments, the head, or the ova in the motions.

(2) **Trematoda.** This order includes one or two species of "flukes" which occur occasionally in man.

(*a*) *Distoma hepaticum* (*Fasciola hepatica*). This, the common liver-fluke of cattle, is not a frequent parasite in man, though it may occur. It is about 30 mm. long, and shaped like a leaf.

(b) *Distoma lanceolatum*. This is a more spindle-shaped fluke, and only one-third the length of the preceding. It also may occur in man. The eggs of both varieties may be found in the stools; those of the former are about 0·12 mm. long, and those of the latter one-third of that. Both are oval, and open at one end by a little lid.

(c) Other varieties of *Distoma*, e.g. *D. crassum* vel *Buski* have been met with (rarely) in man.

(d) The *Bilharzia hæmatobium* is also a fluke, but does not infest the intestinal canal though it probably enters the system by this channel. It is found in the portal vein, and in the veins of the mesentery, kidney, and bladder (see under Urine, chap. viii.).

(3) **Nematoda**. The members of this class are round, as opposed to the flat worms just considered.

(a) *Oxyuris vermicularis*. This, the ordinary thread-worm, is one of the commonest intestinal parasites. They inhabit the colon and rectum, and may often be observed in the motions like little clippings of white thread. The male is the smaller—about 4 mm. in length; the female is 10 mm. long and has a sharp pointed extremity.

(b) *Ascaris lumbricoides*. This is another common parasite, frequently called the 'round worm.' The male is usually 25 cm. (10 in.) in length, the female half as long again. In form they are cylindrical, with pointed extremities, and are yellow-brown in colour. Their habitat is the small intestine, and usually not more than one or two are present at a time, though many may exist in some cases. Some of the French clinicians hold that they give off some irritant poison which may affect the patient constitutionally, producing what has been called

'typho-lumbricosis.' When one worm has been passed, and doubt exists as to the presence of a second, the point can generally be cleared up by microscopic search of the fæces for the ova, which are usually discharged in large numbers. The eggs are brownish-yellow in colour, spheroidal in shape, and have an albuminous coating, a tough, often roughened casing, and granular contents.

(c) *Ascaris mystax*. This is a round worm which inhabits the intestine of the cat, and occasionally is met with in children. The male is 4-6 cm. (2-3 in.) long, and the female 10-12 cm. (4-6 in.). The eggs are quite spherical.

(d) *Tricocephalus dispar*. This parasite, also called the 'whip-worm,' is a comparatively innocuous variety. It derives its popular name from the anterior part of the body being slender and like a whip-lash. Its average length is 5 cm. (2 in.) more or less, the female being a little longer than the male. The eggs are ovoid, and present a peculiar appearance, each end being provided with a lid or cover which juts out as a small prominence.

(e) *Anchylostomum duodenale* (*Dochmius vel Strongylus duodenalis*). This is an important parasite worm, averaging 8-12 mm. ($\frac{1}{3}$ to $\frac{1}{2}$ in.) in length, which has its habitat in the jejunum. It is armed with toothlike hooks, and is a recognised cause of severe anæmia (Egyptian chlorosis) in many tropical climates, as well as in Italy and Switzerland, especially among miners and brick-makers. The male has a tri-lobed expansion or pouch at the tail. The eggs have a thin transparent shell, and the contents may often be seen in the morula stage of development.

(f) *Anguillula intestinalis*. This small worm, 1-2 mm. in length, occurs in the motions in cases of diarrhœa in some tropical countries. It is not known to be of any special pathological importance.

(g) *Trichina spiralis*. The embryos of this parasite find their way into the system from the intestine, and develop in the muscles, where the worm may be found coiled up in a sort of chamber. The mature worm may occasionally be passed in the motions after it has discharged its embryos. The female is 3 mm. long, the male half that size. They are the cause of the disease named "trichiniasis."

Chemical examination of the fæces. This is somewhat limited in its scope as contrasted with the urine and gastric contents, but there are certain tests that may profitably be made use of.

(1) *Blood*. It may not be easy to distinguish the corpuscles in the motions, as they are very apt to get distorted or destroyed. In such a case valuable information may be got from the spectroscope, which reveals the spectra of methæmoglobin and hæmatin, the former showing four bands in acid or neutral solution, the latter one, in alkaline (see fig. 8). On account, however, of the number of derivatives of blood-pigment that may be present, the spectra are apt to be confused. In this case a test of service is the formation of Teichmann's (hæmin) crystals, which can be performed with a small particle of the fæces after the manner described on p. 99).

(2) *Bile-pigments*. These do not occur as such in the fæces in health, but may be found in various morbid conditions. They communicate a green colour to the motions,

and if the latter are alkaline, the pigments tend to be in solution; if acid, in suspension. Gmelin's test may be applied either to a watery extract or to the residue.

(3) *Stercobilin or urobilin*. These are practically identical, and constitute the form in which the bile-pigments occur in the stools.

Stercobilin can be readily extracted from the fæces by macerating a little of the fresh motion with 10 c.cm. of absolute alcohol to which a little acetic acid has been added, and filtering after a short interval. The filtrate is a reddish-brown alcoholic solution of stercobilin, which gives a beautiful green fluorescence with a few drops of 5 per cent. alcoholic solution of zinc chloride and a little ammonia, and which also exhibits spectroscopically the absorption-bands of urobilin.

(4) *Cholesterin*. The crystals, rhombic tables (fig. 16), may be recognised by the microscope; they are soluble in ether, and the addition of a little strong sulphuric acid to them eats them away at the edges with the development of a red colour.

(5) *Mucin*. This is constantly present and may be extracted from fæces owing to its solubility in lime-water. To do this, stir up some of the motions with water, add an equal volume of lime-water, and allow to stand for four hours. On filtering, a solution of mucin will be obtained giving a precipitate with acetic acid.

(6) *Albumin*. This is not normally present in the fæces, but may appear in disease of the bowel. To test for it, the motions should be stirred up with excess of water faintly acidulated with acetic acid. This must be

filtered several times and the filtrate tested for albumin by the tests described on p. 62. Peptones are absent from the fæces in health, but may occur in cases of typhoid fever, dysentery, and tubercular ulceration.

BIBLIOGRAPHY.

The works on general Physiology, Physiological Chemistry, and Medical Diagnosis, mentioned on p. 139, will be found of value, as well as those on Bacteriology (p. 182). In addition the student may consult *The Parasites of Man* (R. Leuckart), Eng. trans. by Wm. Hoyle.

SECTION VI.

PATHOLOGICAL FLUIDS.

Exudates—Pleural effusion—Transudates—Ascitic fluid—Cyst contents — Hydatid — Ovarian — Parovarian — Dermoid — Hydronephrosis—Pancreatic—Cerebro-spinal fluid.

IN many cases where fluid has been withdrawn from the body by aspirating or other means, a chemical analysis will throw much light on its nature, on the circumstances under which it was formed, and the organ or tissues in which it was produced. In many cases the exploring needle or trocar is used to obtain a sample, and the fluids procured in this way may be suitably considered in three groups:

(1) **Exudates or inflammatory effusions.** In general characters these may be serous, sero-purulent, hæmorrhagic, purulent, or chylous. The four last mentioned are distinguished largely by their naked-eye appearances, while an ordinary serous exudation, *e.g.* pleural effusion, is clear or opalescent, colourless, slightly yellow, or, it may be, blood-stained. Exudates usually have a specific gravity of over 1018, and contain, as a rule, more proteids than do transudates (4 per cent. and upwards). If left

standing for twenty-four hours, they coagulate and produce a clot. They often show a sediment of red blood cells, leucocytes, and endothelial cells. The proteids present consist of both albumin and globulin, but not of peptone; a trace of dextrose may occur.

Tests. In examining the fluid chemically, it must first be filtered. The specific gravity may, for clinical purposes, be taken with a urinometer. Albumin is readily detected by acidulation with acetic acid and the application of the ordinary tests for proteids (see p. 62). A quantitative estimation may be made by thoroughly precipitating all the proteid present with trichlor-acetic acid, and collecting, drying, and weighing the precipitate.

Sugar may be detected as follows: The fluid is first acidulated and boiled to coagulate albumin, then filtered and evaporated to a small bulk. The tests of Trommer or Fehling may then be applied.

(2) **Transudates or dropsical effusions.** Of these, ordinary ascitic fluid may be cited as an example. It is often difficult to distinguish between them and exudates, but the former have a distinctly lower specific gravity (1010-12) and less percentage of albumin (1.0-2.5) than the latter. They are watery, and colourless, yellow, greenish, or sanious; do not coagulate on standing, and are alkaline in reaction. They contain fewer cellular elements than inflammatory effusions. The proteids present are chiefly albumin and globulin.

Peptones are absent, but traces of sugar are usually found.

(3) **Contents of cysts.** (*a*) *Hydatid cyst.* The fluid from this is clear and watery, of low specific gravity (1006-12), and neutral or alkaline in reaction.

Albumin, if present at all, is very scanty, but traces of a reducing sugar frequently occur. The most abundant solid constituent is common salt, in which the fluid is rich. The only other important element is succinic acid ($C_4H_6O_4$), which may be extracted by ether from the concentrated and acidulated (HCl) fluid.

Microscopically, hooklets, portions of laminated ectocyst, scolices with hooks and suckers, and hæmatoidin crystals may be found (fig. 32).

(b) *Ovarian cyst.* The specific gravity of the content varies, but tends to be high, about 1020; the fluid is frequently turbid, yellow, greenish, or brown in colour, and alkaline in reaction. On standing, the fluid deposits an abundant sediment as a rule, composed mainly of blood corpuscles, and distorted and degenerated epithelial cells of various kinds.

Ovarian cysts usually contain both albumin and globulin, and, in addition, another proteid body almost invariably occurs named *metalbumin* (sometimes called pseudo-mucin). It is this substance which confers stickiness and turbidity on ovarian fluids.

The general characters of metalbumin are, that it is unaltered by boiling, and gives no precipitate with acetic acid; that it gives a stringy precipitate with alcohol; that it yields a reddish colour on boiling with Millon's reagent; and, lastly, that it yields, on boiling with dilute sulphuric acid, a substance that can reduce Fehling's solution.

Since metalbumin is precipitated by alcohol, it can be separated from ovarian fluid by adding to the latter 2-3 volumes of alcohol, and allowing to stand for a day; on

filtering, a precipitate of metalbumin will be left behind, which can be squeezed free of fluid and dissolved in water, where it gives an opalescent solution. This may be subjected to the tests mentioned above.

(c) *Dermoid cyst*. The contents here resemble those of some ovarian cysts, but are almost always thick and dark; microscopically, they usually show hairs and other animal structures, as well as crystals of cholesterin and hæmatoidin.

(d) *Parovarian cyst*. In this case the fluid is usually clear and limpid, almost colourless, and with a low specific gravity (about 1006). It contains but little albumin, and in many ways forms a marked contrast to that obtained from true ovarian cysts.

(e) *Hydronephrosis*. As the fluid here is in reality a diluted urine, we may rely, in part, for diagnosis on the presence or absence of urea and uric acid. The fluid itself is usually watery and pale, but a little blood or pus may be mixed with it, making it turbid and coloured. The specific gravity, as a rule, ranges between 1008 and 1020, and the amount of proteid present is scanty. Urea may be sought for by evaporating a little of the fluid on a slide, adding a drop of strong nitric acid, and examining microscopically for the hexagonal plates of urea nitrate (fig. 2). The discovery of urea does not, however, make the diagnosis absolutely certain, as it may occur in other cysts. If it be very scanty, a large quantity of the fluid may require to be evaporated down to a small bulk.

A little of this concentrated fluid may be treated with strong hydrochloric acid, which will cause uric acid, if

present, to separate out. The microscope may then be used for its detection.

(f) *Pancreatic cysts.* The contents of these cysts are usually watery, and are frequently stained with blood; the specific gravity varies from 1010-1025, and the reaction is alkaline. Microscopically, crystals of cholesterin are almost always present; a little serum-albumin is frequently met with too.

An important point in diagnosis is that such a fluid can, although alkaline, digest albuminous matter, on account of the trypsin it contains. The test can be carried out by adding a little of the fluid to milk or finely-coagulated egg-albumin at 38° C., and thereafter testing for the biuret reaction. The fluid also contains amylopsin (diastatic ferment) and a substance called tryptophan (especially after it has stood a little), which yields a red or violet colour with bromine- or chlorine-water.

(g) *Cerebro-spinal fluid.* This is sometimes obtained by lumbar puncture, and is a thin watery fluid of low specific gravity, containing but little proteid matter. It possesses, in not a few cases, the power of reducing Trommer's or Fehling's solution.

BIBLIOGRAPHY.

Most of the works on Clinical Diagnosis and Physiological and Pathological Chemistry, mentioned on p. 139, will be found to give information on the subject of Pathological Fluids.

SECTION VII.

THE SKIN AND HAIR.

Animal parasites—Pediculi—Acarus scabiei—Demodex folliculorum — Other animal parasites — Vegetable parasites — Tricophyton—Microsporon Audouini—Microsporon furfur—Achorion Schönleinii—Rarer vegetable parasites.

THE range of application of chemical tests in this department of clinical work is very limited, but the microscope often yields information of much value. In a work of this size, nothing will be attempted beyond a short description of the chief vegetable and animal parasites which may infest the skin and hair.

I. **Animal parasites.** (1) *Pediculi*. Three varieties of pediculi are found affecting man, giving rise to the condition termed Pediculosis or Phtheiriasis.

(a) *Pediculus capitis*. This, the common head-louse, is a greyish-white insect a little over 2 mm. in length and 1 in breadth ($\frac{1}{10}$ by $\frac{1}{20}$ in.). It is provided with six legs, placed anteriorly and armed with hooklets. The female is larger than the male. The eggs (also termed 'nits') adhere very firmly to the hairs, and slant upwards away from the hair. They are carried up with it in

its growth, which affords us an indication of the time of infection.

(b) *Pediculus corporis vel vestimentorum*. This, the louse of the body, infests not the skin but the clothing, and only derives nourishment from the former. Its chief habitat is about and between the shoulders. In size it is larger than the *P. capitis*. The colour of the insect is greyish-white, and the eggs are white.

(c) *Pediculus pubis*. This, the so-called 'crab'-louse, is now sometimes named *phthirius*. It is short and oval, yellow in colour, and is provided with six legs, and many bristles on the hinder portion of the body. It adheres very firmly to the hairs; and the eggs are small and easily passed over.

(2) *Acarus vel Sarcoptes scabiei*. The skin affection, named scabies or 'itch,' is due to this little insect, which is a minute tortoise-shaped object, with a slightly projecting head, and eight legs. The female is the larger, and forms a burrow or 'cuniculus' in the skin, where it lays its eggs. The furrow shows as a dark dotted line, 2-12 mm. in length, and ending in a small white dot. It may be split up by a fine-pointed lancet, and the insect discovered at the end of it. These furrows are often situated at the finger-web or on the ulnar aspect of the wrist.

(3) *Demodex vel Acarus folliculorum*. This parasite, sometimes called the 'comedo-mite,' has its habitat in dilated sebaceous glands. It is a worm-like object, consisting of a head, thorax, tapering abdomen, and eight atrophied legs. It is perfectly harmless and requires no fuller description.

(4) Besides these parasites of the skin already named, we may find the *Pulex irritans*, or flea, a familiar enough black object, provided with three pairs of legs, the hind-most pair being specially strong and long; the *Cimex lectularius*, or bed-bug, a flat, reddish-brown insect; the *Pulex penetrans* (chigoe or 'jigger' insect); the *Ixodes*, or tick-insect, and others of less importance.

II. **Vegetable parasites.** (1) *Tricophyton*. This parasite or fungus may attack different parts of the body, especially the hairy parts. On the head it gives rise to *Tinea capitis vel tonsurans* (common ringworm); on the body to *Tinea corporis vel circinata*; on the cheeks and chin to *Tinea barbæ*; and in the groin and axilla to *Tinea cruris*. It exhibits microscopically a mycelium and spores. In ringworm of the body the mycelium shows few spores as compared with tinea affecting the scalp, or the *Tinea versicolor* or *T. favosa*. In *T. capitis* the patches affected are dotted with black stumps where the brittle diseased hairs have broken short. The spores in this case are very numerous and hide the mycelial threads.

Sabouraud,¹ of Paris, has specially studied the morphology of the ringworm parasite, and concludes that at least two distinct varieties occur in tinea affecting the scalp: one with large spores arranged regularly in chains, *T. megalo-sporon*, and one exhibiting small scattered spores, a *microsporon*. It seems doubtful if this latter form is a true *tricophyton* at all. These varieties differ in their life history and in their effects on the hair and skin. Sabouraud holds that the *microsporon* is the common parasite in *tinea tonsurans* in children, especially in long-standing

¹ *Les Annales de Dermatologie*, 1893-94.

and obstinate cases. It grows in the substance of the hair chiefly, and the mycelium shows segments of varying length. This small-spored variety is identical with the *Microsporon Audouini*, and may be suitably designated by that name.

The megalosporon is found in ringworm of the beard and of the smooth parts of the body. It occurs also in those cases of ringworm of the scalp most amenable to treatment. This variety seems to be the same as the *Endothrix* of the hair described by Sabouraud, Colcott Fox,¹ and others. Microscopically, it appears in chain formation, branching dichotomously. It lies altogether inside the hair. A second variety, *Ectothrix*, affects the skin, and also the beard and nails. It lies partly inside, partly outside, the hairs, and has spores of varying size, some very large, others small.

Tinea imbricata is due to another trichophyton with oval spores arranged in chains.

Examination of hairs for ringworm. A suitable patch having been found, a few diseased hairs are removed by a broad-pointed pair of forceps (epilators), and placed on a glass slide with a large drop of chloroform. As the latter dries up it leaves the affected hairs with a very distinct white or chalky appearance. If a little chloroform be dropped on the patch on the head, the hairs containing spores will become white, those still healthy retaining their normal hue. This change of colour is not seen in eczema or in favus.

When it is desired to examine microscopically, some

¹ "The Biology of Ringworm," *Brit. Med. Jour.*, 1898, vol. i., p. 876.

scrapings may be taken with a blunt knife from the patch, and soaked in a drop of solution of caustic potash (10 per cent.) and glycerine, before examining. If a hair be selected, it may first, with advantage, be washed with ether to remove fatty sebaceous material. After this the hair

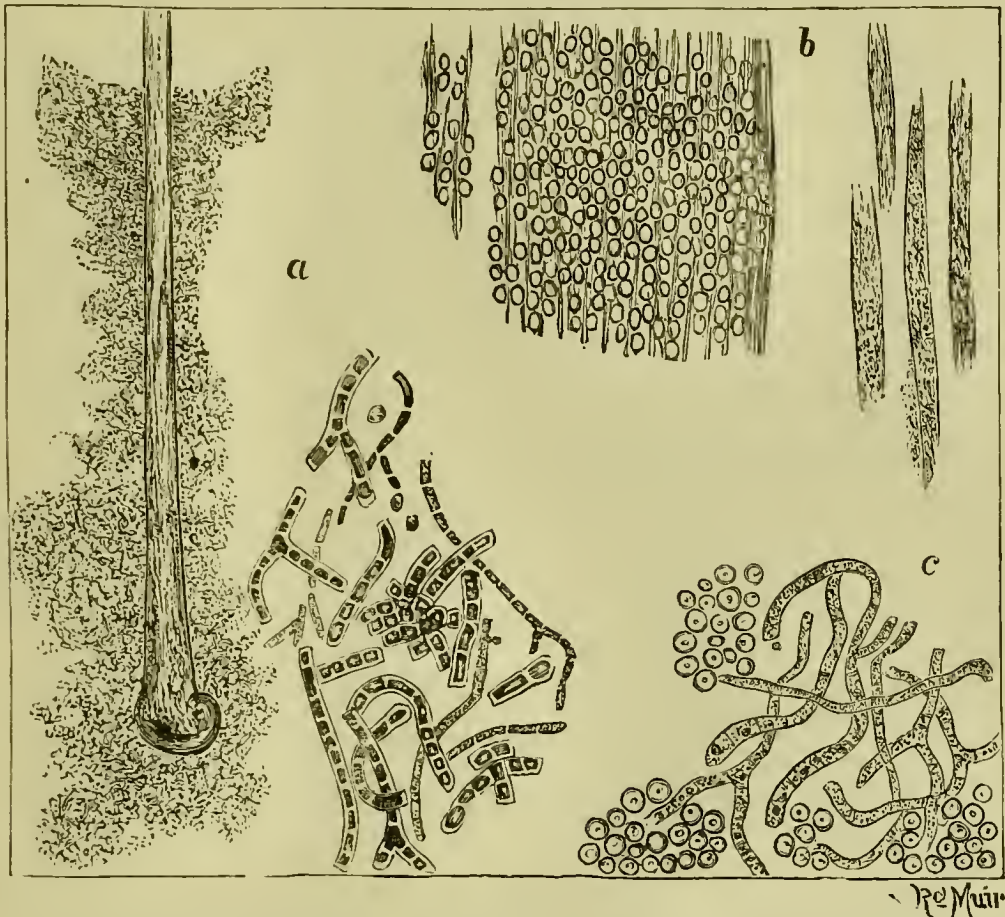


FIG. 33.—VEGETABLE PARASITES OF THE SKIN. *a*, Favus (achorion Schönleinii) showing parasite and affected hair; *b*, ringworm (microsporon Audouini) showing views of affected hairs, high and low power; *c*, pityriasis versicolor, showing microsporon furfur $\times 800$. (Hutchison and Rainy.)

should be soaked for a short time in caustic potash solution, and then examined with a high power lens. The whole breadth of the hair will be found occupied by rows of spores. Mycelial threads will be found more readily in the scrapings than in the hairs (fig. 33). The spores often remain in the hair follicles for a very long time, and after

all the patches seemed healed, a diseased spore-laden stump will be found here and there.

Staining of the fungus. It is sometimes desirable to make stained preparations of the diseased hairs, and this can be done by the method recommended by Morris,¹ the details of which are as follows :

Select and remove an affected hair, and wash it for a few seconds in ether to remove fatty matter. Stain in a solution of gentian violet (5 per cent. in 70 per cent. alcohol) ; the microsporon can stain in five minutes, but the megalosporon may need an hour, including five minutes' heating. If it be intended to make micro-photographs, a red stain should be used in a similar way, *e.g.* a 5 per cent. solution of fuchsin in water containing a little alcohol.

After being stained the hair must be placed in solution of iodine for the purpose of fixing the stain (Gram's solution, p. 175, does very well for this ; let the hair rest in it for ten minutes). The hair is next decolorized by being placed in aniline oil, or in aniline containing a few drops of nitric acid, for ten to fifteen minutes. Finally it is placed in aniline for a few seconds, washed in xylol, and mounted in xylol balsam. In examining use the high power. The chief points of difference between the two varieties are the size of the spores, the regularity with which they are arranged, and the evenness of the jointing of the mycelial threads.

(2) *Microsporon furfur*. This cryptogam is the source of the affection named Tinea versicolor or Pityriasis versicolor. It shows itself by pale yellow, fawn-yellow, or brown areas. Scrapings from these patches, mixed with a little

¹Malcolm Morris, *Ringworm*, 1898, p. 39.

liquor potassæ and glycerine on a slide, and examined microscopically, exhibit the fine branching mycelium with rounded free ends, and the grape-like clusters of spores (fig. 33).

(3) *Achorion Schönleini*. This fungus is the cause of *Tinea favosa* or favus. In its growth there form sulphur-yellow cups (*scutula*), containing yellow crusts having a mouse-like odour. The hairs are harsh, brittle, and dull, but not necessarily broken off short as in ringworm. The microscope shows the spores, and branching mycelial threads with short joints (fig. 33).

(4) Among rarer vegetable parasitic affections may be mentioned erythrasma (due to a microsporon), myringomycosis, actinomycosis of the skin, vaginomycosis, etc.¹

¹See "Parasites of the Skin" (L. D. Bulkely), *Twent. Cent. Pract. of Med.*, 1896.

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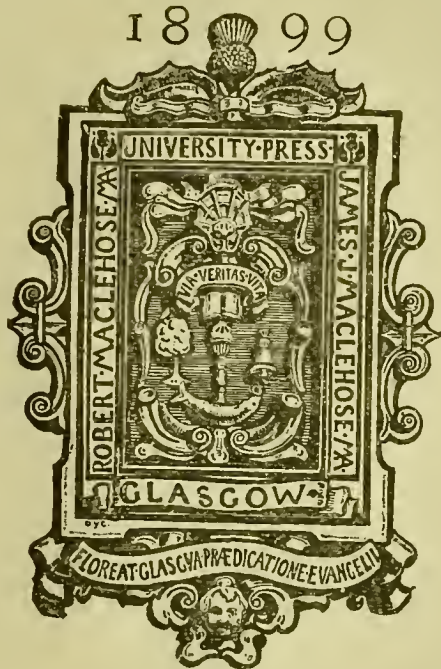
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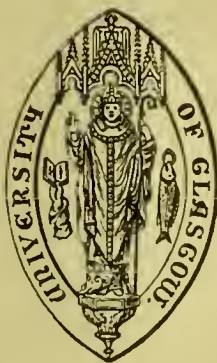


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